A prospective phase 2 study to assess the minimal residual disease after ixazomib plus lenalidomide plus dexamethasone (IRd) treatment for newly diagnosed transplant eligible multiple myeloma patients

Indication: Newly diagnosed transplant eligible multiple myeloma patients

Phase: Phase 2
Version: 4
Date: 28Jan2018

Protocol History:
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Version 2 05 Feb 2017
Version 3 24Mar2017
Version 4 28Jan2018

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Finland
By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/EC procedures, the Declaration of Helsinki, ICH Good Clinical Practices guideline, the EU directive Good Clinical Practice, and local regulations governing the conduct of clinical studies.
**Study Title:** A prospective phase 2 study to assess the minimal residual disease after ixazomib plus lenalidomide and dexamethasone (IRd) treatment for newly diagnosed transplant eligible patients

**Phase:** Phase 2

**Number of Patients:** 120

**Study Objectives**

**Primary**
- To investigate the protocol treatment efficacy based on serological and bone marrow analyses including minimal residual disease assessment by MFC and safety of IRd induction followed by ASCT, IRd consolidation and IR or R maintenance.

**Secondary**
- MRD-negativity at any time during study protocol treatment
- Safety
- Overall response rate (ORR)
- Progression-free survival (PFS) in both groups
- Improvement of responses and MRD negativity during maintenance in both groups
- Time to next treatment
- Quality of life
- Overall survival (OS)

**Tertiary/Exploratory**
- Exome and RNA sequencing of myeloma cells of high-risk patients, drug sensitivity and resistance testing of high-risk patients (translational part of the study with specific funding from Celgene)
- Comparison of MFC and molecular response of MRD-negative patients

**Overview of Study Design:**
This Nordic Myeloma Study Group 23/15 is a first line study for multiple myeloma (MM) patients eligible for high dose treatment (HDT) supported with autologous stem cell transplantation (ASCT). The main aim of this study is to assess the proportion of patients having a MFC-MRD < 0.01% (multiparameter flow cytometry- minimal residual disease < 0.01%), during the protocol treatment including four cycles of IRd induction, single ASCT, two IRd cycles as consolidation and risk stratified maintenance. The total proportion of patients reaching this response will be assessed after one year of maintenance. This is not a
randomized study but we have the Finnish Myeloma Study-MM02 as a historical control with the design of lenalidomide plus bortezomib and dexamethasone (RVD) + single ASCT + lenalidomide maintenance design (NCT01790737).

The present study is using more sensitive MFC method so in addition to the MRD < 0.01% level (comparable with previous MFC-MRD) the secondary end point is MFC-MRD negativity. We assume that treatment with 2nd generation proteasome inhibitor (PI), ixazomib, combined with lenalidomide and dexamethasone during induction and consolidation followed by maintenance will produce higher proportion of patients with low- or negative MRD load. Prolonged treatment with PIs is considered to be important for high-risk-patients, at least for t(4;14) and del17p patients and the risk stratified maintenance phase is planned to answer the question whether the PFS of high-risk and standard-or low-risk patients will be comparable with this design.

**Duration of Study:**

Expected duration of treatment:

- induction 4-5 months
- stem cell mobilization, collection and ASCT with post-transplant supportive care 2 months
- consolidation 2 months, starting within 100 days after ASCT
- maintenance will be started one month after consolidation continuing until progression or toxicity
- The first analyses of the study will be done when the last patient has been two years on maintenance treatment. The treatment of each individual patient will continue until progression or excess toxicity.
- All patients will be followed until 10 years after registration
STUDY OVERVIEW DIAGRAM

3-drug induction
IRd x 4
ASCT eligible

G-CSF or CY+G-CSF
mobilization + harvest

At least PR before ASCT

Single ASCT

CONSOLIDATION
IRd x 2

Primary endpoint:
MFC-MRD < 0.01% achieved at any time
during study protocol

Risk-based stratification

High-risk patients del 17p
(t4;14), t(14;16), t(14;20), +1q
Combined IXA + LEN until PD

Standard + low risk patients
LEN alone until PD
**SCHEDULE OF EVENTS**

**SCHEDULE OF STUDY INVESTIGATIONS**

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* Pregnancy testing should also be performed at the beginning of each 28-day cycle and at the end of study visit, applying to women of childbearing potential
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LIST OF ABBREVIATIONS AND GLOSSARY OF TERMS

Common abbreviations used in oncology protocols are provided below. Program-specific or protocol-specific abbreviations must be added to this list, and unnecessary abbreviations removed, as applicable. Abbreviations that are retained should not be changed.

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<td>5-hydroxytryptamine 3 serotonin receptor</td>
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<td>ALP, AFOS</td>
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<td>alanine aminotransferase</td>
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<td>acute myelogenous leukemia</td>
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<td>area under the plasma concentration versus time curve</td>
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<td>area under the plasma concentration versus time curve from zero to 24 hours</td>
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<td>breast cancer resistance protein</td>
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</tr>
<tr>
<td>ITT</td>
<td>intent-to-treat</td>
</tr>
<tr>
<td>IV</td>
<td>intravenous; intravenously</td>
</tr>
<tr>
<td>KPS</td>
<td>Karnofsky Performance Status</td>
</tr>
<tr>
<td>LDH, LD</td>
<td>lactate dehydrogenase</td>
</tr>
<tr>
<td>LR</td>
<td>Low-risk</td>
</tr>
<tr>
<td>MDS</td>
<td>Myelodysplasia</td>
</tr>
<tr>
<td>MedDRA</td>
<td>Medical Dictionary for Regulatory Activities</td>
</tr>
<tr>
<td>MFC</td>
<td>Multiparameter flow cytometry</td>
</tr>
<tr>
<td>Millennium</td>
<td>Millennium Pharmaceuticals, Inc., and its affiliates</td>
</tr>
<tr>
<td>MM</td>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>MRD</td>
<td>Minimal residual disease</td>
</tr>
<tr>
<td>MTD</td>
<td>maximum tolerated dose</td>
</tr>
<tr>
<td>NCI</td>
<td>National Cancer Institute</td>
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<tr>
<td>NCI CTCAE</td>
<td>National Cancer Institute Common Terminology Criteria for Adverse Events</td>
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<td>NGS</td>
<td>Next generation sequencing</td>
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<tr>
<td>NMSG</td>
<td>Nordic Myeloma Study Group</td>
</tr>
<tr>
<td>nCR</td>
<td>Near complete remission</td>
</tr>
<tr>
<td>NYHA</td>
<td>New York Heart Association</td>
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<tr>
<td>ORR</td>
<td>Overall response rate</td>
</tr>
<tr>
<td>OS</td>
<td>Overall survival</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>PD</td>
<td>Progressive disease</td>
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<tr>
<td>PFS</td>
<td>Progression-free survival</td>
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<td>PB</td>
<td>Peripheral blood</td>
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<td>Pgp</td>
<td>P-glycoprotein</td>
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<tr>
<td>PK</td>
<td>pharmacokinetic(s)</td>
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<tr>
<td>Abbreviation</td>
<td>Term</td>
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<tr>
<td>--------------</td>
<td>------</td>
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<tr>
<td>PO</td>
<td><em>per os</em>; by mouth (orally)</td>
</tr>
<tr>
<td>PR</td>
<td>partial remission</td>
</tr>
<tr>
<td>PRO</td>
<td>patient-reported outcome</td>
</tr>
<tr>
<td>QD</td>
<td><em>quaque die</em>; each day; once daily</td>
</tr>
<tr>
<td>QID</td>
<td><em>quater in die</em>; 4 times a day</td>
</tr>
<tr>
<td>QOD</td>
<td><em>quaque altera die</em>; every other day</td>
</tr>
<tr>
<td>QOL</td>
<td>quality of life</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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<td>SAE</td>
<td>serious adverse event</td>
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<tr>
<td>SC</td>
<td>Subcutaneous</td>
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<tr>
<td>sCR</td>
<td>Stringent complete remission</td>
</tr>
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<td>SD</td>
<td>stable disease</td>
</tr>
<tr>
<td>SJS</td>
<td>Stevens-Johnson syndrome</td>
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<tr>
<td>SmPC</td>
<td>Summary of Product Characteristics</td>
</tr>
<tr>
<td>SPM</td>
<td>Second primary malignancy</td>
</tr>
<tr>
<td>SR</td>
<td>Standard-risk</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt;</td>
<td>terminal disposition half-life</td>
</tr>
<tr>
<td>TEN</td>
<td>Toxic epidermal necrolysis</td>
</tr>
<tr>
<td>TFR</td>
<td>Tumor flare reaction</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>single-dose time to reach maximum (peak) concentration</td>
</tr>
<tr>
<td>TTNT</td>
<td>Time to next treatment</td>
</tr>
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<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>ULN</td>
<td>upper limit of the normal range</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
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<tr>
<td>VTE</td>
<td>Venous thromboembolism</td>
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<tr>
<td>WBC</td>
<td>white blood cell</td>
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<td>WHO</td>
<td>World Health Organization</td>
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1. BACKGROUND AND STUDY RATIONALE

1.1 Scientific background

Multiple myeloma (MM) is the most common hematological malignancy after lymphomas. In years 2009-2013, 955 male and 781 female new myeloma patients per year have been registered in the Nordic Cancer Registries (NORDCAN) giving an incidence of 4.6/100,000 per year.\(^2\) Multiple myeloma is considered as an incurable disease. The overall survival (OS) of patients has doubled in the last 30 years from a median of 2–3 years to 4–6 years in elderly patients, and up to 8–10 years for younger patients, due to autologous stem cell transplantation (ASCT) combined with immunomodulatory drugs and proteasome inhibitors.\(^3\) The International Myeloma Working Group (IMWG) recently proposed new risk stratification standards for MM patients: high-risk (HR), standard-risk (SR) and low-risk (LR) groups, t(4;14) and del(17p) indicating the worst prognosis among cytogenetic aberrations.\(^4\) Although a median OS of LR patients is > 10 years from diagnosis, new drugs and therapeutic innovations are urgently needed for HR patients with a median OS of only two years.\(^4\) High-dose therapy supported by ASCT is the standard therapy for MM in eligible patients under 65 (-70) years.\(^3\) In Nordic countries the upper age limit has been 70 years of age for eligible patients. The general consensus for induction treatment is a triple combination of proteasome inhibitor (PI) plus immunomodulatory, alkylating agent or doxorubicin with dexamethasone for 3-4 cycles.\(^5\) Of the second generation PIs intravenously used carfilzomib has been approved by FDA and EU for MM, ixazomib so far by FDA but some study results have also been published regarding ixazomib for newly diagnosed MM (NDMM).\(^6-16\)

Ixazomib has been now investigated in three phase 1-2 trials for RRMM,\(^6-8\) and in four phase 1-2 trials for NDMM\(^9-16\) with lenalidomide and dexamethasone or melphalan and prednisone and maintenance with lenalidomide, respectively. The maximum tolerated dose was explored to be 2.23mg/m\(^2\) in twice weekly\(^6\) and 2.97mg/m\(^2\) in weekly administration.\(^7\) Dose limiting toxicities were nausea, vomiting, diarrhea, thrombopenia, neutropenia, rash and fatigue. Drug-related peripheral neuropathy was reported from 12% to 20% with only one grade 3.\(^6-7\) 15%-18% of patients achieved PR or better with ixazomib monotherapy.\(^6-7\) Stem cell mobilization after median of 4 (3-9) cycles of IRd resulted in the median number of 11.6 (5-28) x 10\(^6\)/kg CD34\(^+\) cells.\(^12\) In phase 3 study (Tourmaline-MM1) IRd increased the median PFS to 20.6 from 14.7 months of Rd (HR 0.742, p=0.012) without increase of overall toxicity and also del17p patients achieved a similar benefit. ORR was 78.3% compared to 71.5% (p=0.035) with ≥ VGPR rate of 48.1% and 39.0% (p=0.014).\(^17\) The rate of ≥ grade 3 AEs were 68% vs. 61% (thrombocytopenia most common). Most important adverse events were gastrointestinal (42% vs. 36%), polyneuropathy (28% vs. 21%) (2% vs. 2% gr 3), rash (35% vs. 21%) (4% vs. 1% gr 3).\(^16\)
The importance of attaining the traditional complete response of ASCT for OS is confirmed in a meta-analysis. Several studies have demonstrated also the correlation between low or negative minimal residual disease (MRD) after treatment and improved outcome in MM and in recent years several publications have confirmed the impact of MRD-negative status for long-term outcome. FDA has accepted the MRD as a new criteria for assessing outcome in addition to PFS and OS. Both of allele specific real-time quantitative polymerase chain reaction, ASO RQ-PCR, multiparameter flow cytometry (MFC) and next generation sequencing (NGS) have been used in MM to assess MRD. The MFC and ASO RQ-PCR have been compared with NGS assay for MRD assessment and NGS seems to be most sensitive bypassing the technical problems related to designing the PCR probe, but this has to be confirmed. To standardize the MFC method after the first paper of Rawstron et al. EuroFlow Consortium has published the guidelines for MFC for diagnostic panel in plasma cell disease. The consensus guidelines for MFC-MRD sample processing and MRD detection have now been published. In practice MFC is the most practical and fastest method for MRD assessment but in studies with new generation myeloma drugs NGS has been considered for comparison with this new MFC-MRD panel. NGS might be able to identify the clones left after study protocol treatment in different patient groups and to identify biomarkers correlating with outcome.

In the Nordic countries about 400 upfront ASCTs are performed every year in MM. However, ASCT is not considered curative, and most patients will progress within 2-3 years. Therefore post-ASCT therapy, consolidation and maintenance are widely investigated to improve the depth of response and to achieve sustained long-term response and remission. So far very limited data has been published regarding MRD after this new IRd induction and until now there is no data concerning MRD data after IRd followed by ASCT and IRd consolidation. Oral treatment would be most convenient for patients and would decrease hospital visits and costs.

This Nordic Myeloma Study Group study is a first-line study for transplant-eligible MM patients below 70 years of age. The main aim of this study is to assess the proportion of patients having a MFC-MRD < 0.01% at any time during the study protocol treatment measured after one year of maintenance treatment. This is not a randomized study but we have the Finnish Myeloma Study-MM02 as a historical control with the design of lenalidomide plus bortezomib and dexamethasone + single ASCT + lenalidomide maintenance design (NCT01790737). The present study is using more sensitive MFC method so in addition to the MRD < 0.01% level (comparable with previous MFC-MRD) the secondary endpoint is MFC-MRD negativity. We assume that treatment with 2nd generation PI + IMiD + dexamethasone including in induction and consolidation followed by maintenance will produce higher proportion of patients with low- or negative MRD status. Prolonged treatment with PIs is considered to be important for HR-
patients, at least for t(4;14) and del17p patients and the risk stratified maintenance phase is planned to answer the question whether the PFS of HR and LR/SR patients will be comparable with this design.

1.1.1 Disease Under Treatment

Multiple myeloma without prior treatment

1.2 Ixazomib (MLN9708)

1.2.1 Preclinical Experience

Referring to the current ixazomib Investigator’s Brochure (IB) and Safety Management Attachment (SMA).

1.2.2 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, 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,
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 7.9) 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.
1.2.3 Pharmacokinetics and Drug Metabolism

After oral dosing, absorption of ixazomib is rapid with a median first time to maximum observed plasma concentration ($T_{\text{max}}$) 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 $C_{\text{max}}$ 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 $C_{\text{max}}$ 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 ($\text{CrCL} \geq 30 \text{ mL/min}$) 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.

1.2.4 Clinical Trial Experience Using the Oral Formulation of Ixazomib

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 is included in Table 1-1.
### Table 1-1  Clinical Studies of Oral Ixazomib

<table>
<thead>
<tr>
<th>Trial/Population</th>
<th>Description</th>
<th>Doses Investigated</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16003 RRMM</td>
<td>PO, TW, single agent</td>
<td>0.24-2.23 mg/m² TW</td>
</tr>
<tr>
<td>N = 60</td>
<td></td>
<td>MTD: 2.0 mg/m²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DLT: rash, thrombocytopenia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Closed to enrollment</td>
</tr>
</tbody>
</table>

| C16004 RRMM      | PO, W, single agent | 0.24-3.95 mg/m² W  |
| N = 60           |              | MTD: 2.97 mg/m²     |
|                  |              | DLT: rash, nausea, vomiting, diarrhea |
|                  |              | Closed to enrollment |

| C16005 NDMM      | PO, W, combination with LenDex | 1.68-3.95 mg/m² W  |
| N = 65           | 28-day cycle | MTD: 2.97 mg/m²     |
|                  |              | DLT: nausea, vomiting, diarrhea, syncope |
|                  |              | RP2D: 4.0 mg fixed (switched to fixed dosing in phase 2, equivalent to 2.23mg/m²) |
|                  |              | Closed to enrollment |

| C16006 NDMM      | PO, TW (Arm A: 42 day cycle) and W (Arm B: 28 day cycle), combination with Melphalan and Prednisone | Arm A: 3-3.7-mg fixed dose TW |
| N = 20           |              | DLT: rash, thrombocytopenia, subileus |
|                  |              | Arm B: 3-5.5-mg fixed dose, W |
|                  |              | DLT: Esophageal ulcer nausea, vomiting, hematemesis, thrombocytopenia, ileus, neurogenic bladder |
|                  |              | MTD = 3.0 mg |
|                  |              | Closed to enrollment |

| C16007 RRAL      | PO, W, single agent | 4-5.5-mg fixed dose W  |
| N = 27           |              | DLT: thrombocytopenia, diarrhea, dyspnea, acute rise in creatinine, cardiac arrest |
|                  |              | MTD: 4.0 mg W  |

| C16008 NDMM      | PO, TW, combination with LenDex | 3.0-3.7-mg fixed dose W  |
| N = 64           | 21-day cycle | MTD: 3.0 mg  |
|                  |              | Closed to enrollment |

| C16009 RRMM      | PO, W, single agent | 5.5-mg fixed dose W  |
| N = 9            |              |                   |

| C16010 RRMM      | PO, W, with LenDex versus placebo-LenDex | 4.0 mg W  |
| N = 200          |              |                   |

| C16011 RRAL      | PO, W, with Dex versus physician’s choice of a Dex-based regimen | 4.0 mg W  |
| N = 4            |              |                   |

| C16013 RRMM      | PO, W, with LenDex | 4.0 mg W  |
| N = 9            |              |                   |
### Table 1-1  Clinical Studies of Oral Ixazomib

<table>
<thead>
<tr>
<th>Trial/Population</th>
<th>Description</th>
<th>Doses Investigated</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16014</td>
<td>Symptomatic MM, N=701, PO, combination with LenDex</td>
<td>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 &gt;75 years old) on Days 1, 8, 15, and 22</td>
</tr>
<tr>
<td>C16015</td>
<td>Symptomatic MM with normal renal function or severe renal impairment, N=28, PO, combination with Dex</td>
<td>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 &gt;75 years old) on Days 1, 8, 15 and 22 of a 28-day cycle</td>
</tr>
<tr>
<td>C16017</td>
<td>RR follicular lymphoma, N=58, PO, W</td>
<td>4.0, 5.3, and 7.0 mg, W Treatment at RP2D once determined.</td>
</tr>
<tr>
<td>C16018</td>
<td>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</td>
<td>1.5 mg (severe hepatic impairment), 2.3 mg (moderate hepatic impairment), or 4.0 mg (normal hepatic function)</td>
</tr>
<tr>
<td>TB-MC010034</td>
<td>RRMM, N = 10, PO, W</td>
<td>4.0 mg, W Single agent: 4.0 mg Combination with Rd</td>
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</tbody>
</table>

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/m² BSA dosing; 4.0 mg ~ equivalent to 2.23 mg/m² BSA dosing; and 5.5 mg~ equivalent to 2.97 mg/m² BSA dosing.
Overview of the Oral Formulation of Ixazomib

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 in Table 1-2.

### Table 1-2 Most Common (At Least 10% of Total) Treatment-Emergent Adverse Events in Oral Single-Agent Studies

<table>
<thead>
<tr>
<th>Primary System Organ Class</th>
<th>Oral Single Agent Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preferred Term</td>
<td>n = 201 n (%)</td>
</tr>
<tr>
<td>Subjects with at Least One Adverse Event</td>
<td>197 (98)</td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
<td>160 (80)</td>
</tr>
<tr>
<td>Nausea</td>
<td>106 (53)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>88 (44)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>77 (38)</td>
</tr>
<tr>
<td>Constipation</td>
<td>46 (23)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>33 (16)</td>
</tr>
<tr>
<td>General disorders and administration site conditions</td>
<td>151 (75)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>103 (51)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>51 (25)</td>
</tr>
<tr>
<td>Oedema peripheral</td>
<td>27 (13)</td>
</tr>
<tr>
<td>Asthenia</td>
<td>31 (15)</td>
</tr>
<tr>
<td>Nervous system disorders</td>
<td>92 (46)</td>
</tr>
<tr>
<td>Headache</td>
<td>29 (14)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>26 (13)</td>
</tr>
</tbody>
</table>
As of 04 Jan 2016, there are 14 studies actively enrolling patients with multiple myeloma to investigate oral ixazomib in combination with different combination regimens and one phase 2 study evaluating bone remodelling during ixazomib treatment.

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 1-3). Note that in combination trials, related is defined as related to any study drug in the combination regimen.

<table>
<thead>
<tr>
<th>Primary System Organ Class</th>
<th>Oral Single Agent Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preferred Term</td>
<td>n = 201</td>
</tr>
<tr>
<td>Neuropathy peripheral</td>
<td>21 (10)</td>
</tr>
<tr>
<td>Metabolism and nutrition disorders</td>
<td>107 (53)</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>64 (32)</td>
</tr>
<tr>
<td>Dehydration</td>
<td>37 (18)</td>
</tr>
<tr>
<td>Blood and lymphatic system disorders</td>
<td>98 (49)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>68 (34)</td>
</tr>
<tr>
<td>Anaemia</td>
<td>42 (21)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>29 (14)</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>20 (10)</td>
</tr>
<tr>
<td>Skin and subcutaneous tissue disorders</td>
<td>90 (45)</td>
</tr>
<tr>
<td>Rash macular</td>
<td>23 (11)</td>
</tr>
<tr>
<td>Musculoskeletal and connective tissue disorders</td>
<td>93 (46)</td>
</tr>
<tr>
<td>Back pain</td>
<td>24 (12)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>28 (14)</td>
</tr>
<tr>
<td>Respiratory, thoracic and mediastinal disorders</td>
<td>78 (39)</td>
</tr>
<tr>
<td>Cough</td>
<td>28 (14)</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>30 (15)</td>
</tr>
<tr>
<td>Infections and infestations</td>
<td>89 (44)</td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>31 (15)</td>
</tr>
</tbody>
</table>

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.

Note that rash maculopapular and rash macular represent the 2 most common terms used to describe rash.
## Table 1-3  Most Common (At Least 10% of Total) Treatment-Emergent Adverse Events in Oral Combination Studies

<table>
<thead>
<tr>
<th>Primary System Organ Class</th>
<th>Total Oral Combo Agent (5/6/8/13)</th>
<th>n (%)</th>
<th>Preferred Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects with at least one adverse event</td>
<td>163 (94)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
<td>139 (80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>65 (38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>81 (47)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>51 (29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td>57 (33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>General disorders and administration site conditions</td>
<td>132 (76)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>76 (44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrexia</td>
<td>39 (23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oedema peripheral</td>
<td>61 (35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthenia</td>
<td>20 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nervous system disorders</td>
<td>115 (66)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>28 (16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td>34 (20)</td>
<td></td>
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</tr>
<tr>
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<td></td>
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<tr>
<td>Metabolism and nutrition disorders</td>
<td>91 (53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>25 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypokalaemia</td>
<td>34 (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood and lymphatic system disorders</td>
<td>88 (51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>49 (28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaemia</td>
<td>45 (26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutropenia</td>
<td>43 (25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>20 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin and subcutaneous tissue disorders</td>
<td>102 (59)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash maculopapulara</td>
<td>29 (17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash maculara</td>
<td>22 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musculoskeletal and connective tissue disorders</td>
<td>99 (57)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Back pain</td>
<td>42 (24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain in extremity</td>
<td>31 (18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthralgia</td>
<td>22 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory, thoracic and mediastinal disorders</td>
<td>80 (46)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>36 (21)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 1-3 Most Common (At Least 10% of Total) Treatment-Emergent Adverse Events in Oral Combination Studies

<table>
<thead>
<tr>
<th>Primary System Organ Class</th>
<th>Total Oral Combo Agent (5/6/8/13)</th>
<th>n = 173</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyspnoea</td>
<td></td>
<td>26</td>
<td>(15)</td>
</tr>
<tr>
<td>Infections and infestations</td>
<td></td>
<td>92</td>
<td>(53)</td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td></td>
<td>35</td>
<td>(20)</td>
</tr>
<tr>
<td>Psychiatric disorders</td>
<td></td>
<td>73</td>
<td>(42)</td>
</tr>
<tr>
<td>Insomnia</td>
<td></td>
<td>50</td>
<td>(29)</td>
</tr>
</tbody>
</table>

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.

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

The clinical experience with ixazomib 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 ixazomib, when combined with established therapies, and across the malignancies studied (advanced solid tumors, non-Hodgkin’s disease, Hodgkin’s disease, relapsed and/or refractory multiple myeloma [RRMM; 42-43], relapsed or refractory systemic light chain amyloidosis [RRAL; 44], and newly diagnosed multiple myeloma [NDMM; 45-47]) to date.

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

#### 1.2.5 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. 48-49 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. 50-52 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. Referring to the ixazomib IB and SMA for further information.

The first analysis of 3 TOURMALINE-MM 1 phase 3 study (NCT01564537) demonstrated a 35% improvement in PFS with the combination of ixazomib plus lenalidomide and dexamethasone (IRd) (n=360) compared to placebo plus lenalidomide and dexamethasone (placebo-Rd) (n=362) for patients with RRMM. The median PFS was 20.6 and 14.7 months, for IRd and Rd respectively. TTP and response rates were also significantly improved with IRd combination. The addition of ixazomib combined to Rd increased median PFS without a substantial increase in overall toxicity, including cardiac toxicity and peripheral neuropathy. In patients with high-risk cytogenetics, the PFS HR was 0.543 with IRd vs Rd (HR 0.596 in patients with del(17)), with a median PFS similar to the overall IRd group, indicating ixazomib may overcome the negative impact of cytogenetic alterations.

1.2.6 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). Referring to the ixazomib IB for further information.

In addition, three studies for NDMM are ongoing, C16019 ixazomib vs placebo maintenance after ASCT, C16020 (NCT02046070) ixazomib + cyclophosphamide + dexamethasone (ICd) for NDMM not transplant eligible and C16021 ixazomib vs placebo maintenance for transplant ineligible. The first results of C16020 phase 2 trial were presented at ASH 2015 showing high ORRs with manageable toxicity with this combination without IMiDs. ICd with 300 mg dose of cyclophosphamide maybe a more preferable regimen for elderly NDMM patients.53

1.2.7 Clinical Trial Experience Using the Intravenous Formulation of Ixazomib

See the IB for descriptions of the 2 studies that investigated IV ixazomib in advanced solid tumors and advanced lymphoma (Studies C16001 and C16002, respectively).

1.3 Lenalidomide

Lenalidomide is a thalidomide analogue, an immunomodulatory agent with anti-angiogenic properties. The chemical name is 3-(4-amino-1,3-dihydro-1-oxo- 2H-isooindol-2-yl) piperidine-2,6-dione. Lenalidomide is rapidly absorbed under fasting condition following oral administration, with the Cmax in plasma usually occurring at a median time of approximately 0.5 and 1.5 hours postdose. In the pivotal efficacy and safety MM registration trials the drug was administered without regard to food intake. Thus, lenalidomide can be administered with or without food. Lenalidomide is not a substrate of hepatic metabolic enzymes in vitro and metabolism contributes to a very minor extent to the systemic clearance of lenalidomide in humans. Lenalidomide is eliminated predominantly through renal excretion of the unchanged drug. Approximately 65%-85% of the administered dose is excreted in urine in unchanged drug. The elimination half-life is appr. 3 to 5 hours at the clinically relevant doses. Steady-state levels are achieved within 4 days. Lenalidomide is not expected to affect dexamethasone metabolism because lenalidomide is not an inducer of the human CYP enzyme. P-gp does not play an important role in pharmacokinetics of lenalidomide in humans.

Lenalidomide European SmPC and sIMPD.

1.3.1 Supplier and dosage form

Celgene Corporation will supply lenalidomide 5 mg, 10 mg, 15 mg, 20 mg and 25 mg capsules free of charge for the duration of this trial and dosage will be administered by the study
Protocol. The drug will be sent to a central pharmacy of each study country. The central pharmacy will be responsible for distributing the drug to the sub-sites. No distribution will take place before required documentation is in place.

1.3.2. Packaging

Celgene Corporation will supply lenalidomide 5 mg, 10 mg, 15 mg and 25 mg capsules in individual and labeled blisters. Celgene Corporation will supply lenalidomide 20 mg capsules in blisters/bottles. Blisters/bottles will contain sufficient drug to last for 21 days of dosing. Study drug must be dispensed in the original packaging with the label clearly visible.

5mg, 10mg, 15mg and 25 mg (and also 20 mg after the initial supply) capsules are in blisters identical to commercial use and provided in wallets. They are labelled for clinical trial use.

Initially 20 mg will be supplied as clinical image in the start of the study. Capsule shell is visually different (white, no markings) from commercial product. They are packaged in bottles/blisters and labelled for clinical trial use.

1.3.3 Lenalidomide Medication Recipient and Storage

The investigator(s) or designee(s) is responsible for taking an inventory of each shipment of lenalidomide received, and comparing it with the accompanying lenalidomide accountability form. The investigator(s) will verify the accuracy of the information on the form, sign and date it, retain a copy in the study file, and return to Celgene. At the study site, all investigational study medications will be stored in a locked, safe area to prevent unauthorized access. The study drug lenalidomide should be stored at room temperature (excursions permitted to +15-+30°C) away from direct sunlight and protected from excessive heat and cold.

1.3.4 Record of Administration

Accurate recording of all study medication administration (including dispensing and dosing) will be made in the appropriate section of the subject’s CRF and source documents.

1.3.5 Lenalidomide Medication Accountability

The investigator(s) or designee(s) is responsible for accounting for all study medication that is issued to and returned by the subject during the course of the study.
1.3.6 Lenalidomide Handling and Disposal

Investigator or designee will return unused study drugs to pharmacy for destruction. If any study medication is lost or damaged, its disposition should be documented in the subject’s CRF and source documents.

1.3.7 Potential adverse effects of lenalidomide

The identified AEs associated with the use of lenalidomide across all studied indications are commonly related to the blood and lymphatic systems, gastrointestinal disorders, infections and infestations, skin and subcutaneous tissue disorders, and vascular disorders. The most serious adverse reactions are venous thromboembolism (deep vein thrombosis, pulmonary embolism) and grade 4 neutropenia.

The most frequently observed adverse reactions which occurred significantly more frequently in the lenalidomide/dexamethasone group compared to the placebo/dexamethasone group were neutropenia (39.4%), fatigue (27.2%), anemia (17.6%), constipation (23.5%), muscle cramp (20.1%), thrombocytopenia (18.4%), anaemia (17.0%), diarrhoea (14.2%) and rash (10.2%).

Hematological toxicity: Neutropenia and thrombocytopenia: Lenalidomide is associated with anemia, neutropenia, febrile neutropenia, thrombocytopenia and pancytopenia. Grade 3 or 4 neutropenia and thrombocytopenia are the most common dose-limiting AEs. A complete blood cell count, including WBC count with differential, platelet count, haemoglobin and haematocrit, should be performed to monitor for cytopenias in accordance with the protocol during the study and cytopenias should be handled by protocol guidelines. Patients with neutropenia should be monitored for signs of infection. Patients and physicians are advised to be observant for signs and symptoms of bleeding, including petechiae and epistaxis, especially in case of concomitant medication susceptible to induce bleeding, a dose reduction of lenalidomide may be required.

Gastrointestinal disorders: Constipation, diarrhea, nausea, and vomiting were the most commonly reported gastrointestinal AEs during treatment with lenalidomide.

Hepatic disorders: Cases of transient liver laboratory abnormalities, predominantly transaminases, were reported in patients treated with lenalidomide. Treatment with lenalidomide should be interrupted and restarted once the levels return to baseline. Successful rechallenge without recurrence of liver laboratory elevations was reported in some patients. In postmarketing surveillance, a few cases of acute hepatic failure, including fatalities, were reported. The mechanism of reported hepatic toxicity remain unknown although, in some cases, pre-existing viral liver disease, elevated baseline liver enzymes, and possibly treatment with antibiotics might be risk factors.
Infections and infestations: Treatment-emergent AEs of infections, specifically pneumonia, are commonly seen with lenalidomide.

Cardiac disorders: Adverse events such as atrial fibrillation, myocardial infarction and heart failure have been reported with the use of lenalidomide from clinical studies and postmarketing surveillance. Patients with prior history and known risk factors for these AEs should be closely monitored.

Venous and arterial thromboembolic events: There is an increased risk of VTEs (predominantly deep vein thrombosis and pulmonary embolism) in MM patients treated with lenalidomide in combination with dexamethasone or other chemotherapy. There is also an increased risk of arterial thromboembolic events (predominantly myocardial infarction and cerebrovascular event). Patients with known risk factors for thromboembolism- including prior thrombosis- should be closely monitored. Action should be taken to try to minimize all modifiable risk factors (eg. smoking, hypertension, hyperlipidemia). Erythropoietin agents and hormone-replacement therapy, should be used with caution in patients receiving lenalidomide and dexamethasone. Patients and physicians are advised to be observant for the signs and symptoms of thromboembolism. Patients should be instructed to seek medical care if they develop symptoms such as shortness of breath, chest pain, arm or leg swelling.

If the patient experiences any thromboembolic events, treatment must be discontinued and standard anticoagulation therapy started. Once the patient has been stabilised on the anticoagulation treatment and any complications of the thromboembolic event have been managed, the lenalidomide treatment may be restarted at the original dose dependent upon a benefit risk assessment. The patient should continue anticoagulation therapy during the course of lenalidomide treatment.

Renal impairment: Since lenalidomide is primarily excreted unchanged by the kidney, starting dose adjustments is recommended in patients with renal impairment. Therefore care should be taken in dose selection and monitoring of renal function is advised in patients with renal impairment (see Table 1-4)

Thyroid function: Cases of hypothyroidism and thyroid dysfunction have been reported and monitoring of thyroid function should be considered.

Peripheral neuropathy: Lenalidomide is structurally related to thalidomide, which is known to induce severe peripheral neuropathy. At this time, the neurotoxic potential of lenalidomide associated with long-term use cannot be ruled out.

Tumour Lysis Syndrome: Because lenalidomide has anti-neoplastic activity the complications of tumour lysis syndrome may occur. The patients at risk of tumour lysis syndrome are those
with high tumour burden prior to treatment. These patients should be monitored closely and appropriate precautions taken.

Allergic Reactions: Cases of allergic reaction/hypersensitivity reactions have been reported.

Severe skin reactions: Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) have been reported. Lenalidomide must be discontinued for angioedema, grade 4 rash, exfoliative or bullous rash, or if SJS or TEN is suspected, and should not be resumed following discontinuation for these reactions. Discontinuation of lenalidomide should be considered if skin rash ≥ grade 2 is exfoliative or bullous or if SJS or TEN is suspected. For other forms of skin rash lenalidomide should not be resumed following discontinuation for these reactions.

Musculoskeletal and connective tissue disorders: The rare AE of rhabdomyolysis has been observed with lenalidomide. The reports of rhabdomyolysis were confounded by concurrent use of statins and dexamethasone, concurrent viral and bacterial infections, trauma, and serotonin syndrome, which are risk factors for rhabdomyolysis.

Neoplasms benign, malignant and unspecified. Second primary malignancies: NDMM: In clinical trials of NDMM an increase of invasive SPMs, most notably AML and MDS, has been observed predominantly in subjects receiving lenalidomide in combination with melphalan or immediately following high-dose melphalan and ASCT. The incidence rate was 1.57 per 100 person-years for the combined MPR arms and 0.36 per 100 person-years for the MPP+p control arm. Cases of B-cell malignancies were observed in clinical trials where subjects received lenalidomide in the post-ASCT setting. Patients should be carefully evaluated before and during treatment using standard cancer screening for occurrence of SPMs, and treatment should be instituted as appropriate.

Postmarketing data: Pneumonitis, transient abnormal liver laboratory tests, hyperthyroidism, hypothyroidism, TLS, TFR and allergic conditions including angioedema, SJS and TEN have been identified and are considered by Celgene to be at least possibly related to lenalidomide.

Lactose intolerance: Revlimid capsules contain lactose. Patients with rare hereditary problems of galactose intolerance, the Lapp lactase deficiency or glucose-galactose malabsorption should not take this medicinal product.

Unused capsules: Patients should be advised never to give this medicinal product to another person and to return any unused capsules to their pharmacist at the end of the treatment.

Lenalidomide is not a substrate of CYP enzymes in vitro, is a weak substrate but not an inhibitor of P-gp. Lenalidomide and concomitant digoxin: Concomitant administration with lenalidomide...
10mg/day increased the plasma exposure of digoxin by 14%. Periodic monitoring of digoxin levels in patients receiving concomitant lenalidomide is recommended based on standard clinical practice. Lenalidomide and concomitant warfarin: Monitoring of warfarin concentration in accordance with standard practice is advised during treatment with lenalidomide.

Because of the increased risk of venous thromboembolism in subjects with multiple myeloma taking lenalidomide and dexamethasone, combined oral contraceptive pills are not recommended. If a subject is currently using combined oral contraception the subject should switch to another one of the highly effective methods listed above. The risk of venous thromboembolism continues for 4 to 6 weeks after discontinuing combined oral contraception. The efficacy of contraceptive steroids may be reduced during co-treatment with dexamethasone.

1.4. **DEXAMETHASONE**

Dexamethasone is not an investigational drug in this study. Commercial supplies of dexamethasone will be utilized according the local routine.

1.4.1 Potential adverse effects of dexamethasone

Allergic Reactions: Anaphylactoid reaction, anaphylaxis, angioedema.

Cardiovascular: Bradycardia, cardiac arrest, cardiac arrhythmias, cardiac enlargement, circulatory collapse, congestive heart failure, fat embolism, hypertension, hypertrophic cardiomyopathy in premature infants, myocardial rupture following recent myocardial infarction, edema, pulmonary edema, syncope, tachycardia, thromboembolism, thrombophlebitis, vasculitis.

Dermatologic: Acne, allergic dermatitis, dry scaly skin, ecchymoses and petechiae, erythema, impaired wound healing, increased sweating, rash, striae, suppression of reactions to skin tests, thin fragile skin, thinning scalp hair, urticaria.

Endocrine: Decreased carbohydrate and glucose tolerance, development of cushingoid state, hyperglycemia, glycosuria, hirsutism, hypertrichosis, increased requirements for insulin or oral hypoglycemic agents in diabetes, manifestations of latent diabetes mellitus, menstrual irregularities, secondary adrenocortical and pituitary unresponsiveness (particularly in times of stress, as in trauma, surgery, or illness), suppression of growth in pediatric patients.
Fluid and Electrolyte Disturbances: Congestive heart failure in susceptible patients, fluid retention, hypokalemic alkalosis, potassium loss, sodium retention.

Gastrointestinal: Abdominal distention, elevation in serum liver enzyme levels (usually reversible upon discontinuation), hepatomegaly, increased appetite, nausea, pancreatitis, peptic ulcer with possible perforation and hemorrhage, perforation of the small and large bowel (particularly in patients with inflammatory bowel disease), ulcerative esophagitis.

Metabolic: Negative nitrogen balance due to protein catabolism.

Musculoskeletal: Aseptic necrosis of femoral and humeral heads, loss of muscle mass, muscle weakness, osteoporosis, pathologic fracture of long bones, steroid myopathy, tendon rupture, vertebral compression fractures.

Neurological/Psychiatric: Convulsions, depression, emotional instability, euphoria, headache, increased intracranial pressure with papilledema (pseudotumor cerebri) usually after treatment, insomnia, mood swings, neuritis, neuropathy, paresthesia, personality changes, psychic disorders, vertigo.

Ophthalmic: Exophthalmos, glaucoma, increased intraocular pressure, posterior subcapsular cataracts.

Other: Abnormal fat deposits, decreased resistance to infection, hiccups, increased or decreased motility and number of spermatozoa, malaise, moon face, weight gain.

Dexamethasone: It may not be excluded that the efficacy of oral contraceptives may be reduced during dexamethasone treatment. Effective measures to avoid pregnancy must be taken.

1.5 Study Rationale

This is a phase II study to test the efficacy and feasibility of this new 3-drug combination, ixazomib plus lenalidomide and dexamethasone (IRd) as induction before ASCT, as consolidation post-ASCT and as risk based stratification on maintenance phase. The efficacy and safety of IRd has already been shown in phase 3 study for relapsed and/or refractory\textsuperscript{17} and in phase 1-2 studies for newly diagnosed MM patients.\textsuperscript{9-16} ASCT is the standard treatment in transplant eligible MM patients < 70 years of age in all Western countries. Two international studies are now ongoing comparing intensive versus non-intensive arm: IFM – DFCI 2009 and HOVON 95 MM.\textsuperscript{54-55} The role of upfront ASCT is so far confirmed with the first study showing 3-year PFS benefit of 61% for ASCT arm vs. 48% for novel agents without upfront ASCT
However, there is a need to deepen the responses before and after ASCT to prolong progression-free and overall survival but without adding toxicity. Patient with standard- or low-risk benefit most from new myeloma drugs but the outcome of high-risk patients is still poor and needs different therapy approach. The risk stratified maintenance phase of this study is aimed to explore that challenge.

The depth of complete remission (CR) and immunophenotypic remission has been shown to be important for outcome, and there are not any studies published aiming to assess the immunophenotypic remission after IRd induction followed by ASCT, IRd consolidation and ixazomib plus lenalidomide or lenalidomide maintenance. In addition, there is no data evaluating the immunophenotypic response to ixazomib plus lenalidomide maintenance in high-risk (HR) patients in order to assess if this is comparable to lenalidomide maintenance in standard risk (SR) or low-risk (LR) patients. IRd will be an costly but oral drug combination and it is important to evaluate the depth and quality of response during treatment. If oral 3-drug treatment combined with upfront ASCT will reach at least similar or even better efficacy than parenterally used comparative drugs, treatment of that combination probably would be preferred in routine practice. The health economy and quality of life analyses will be included in the study.

This study will also evaluate the number of CD34+ cells achieved after 3-4 IRd cycles mobilized with CY+G-CSF or G-CSF alone (plerixafor if needed) by standard routines of sites. If G-CSF mobilization would result with sufficient yield the hospitalization of NDMM patient would be extremely diminished compared to earlier treatment regimens.

### 1.5.1 Translational part of study

As a separative research project CD138+ cells from high-risk patients will be tested in a drug sensitivity and resistance testing (DSRT) assay, which is a functional test to assess the ex-vivo sensitivity of the cells against the panel of approximately 150 small molecule inhibitors including multiple myeloma drugs. In addition, exome sequencing will be done for the collected patient samples of high-risk patients. These assays will be done as part of a separately funded and designed project (Celgene/CITRE/Institute for Molecular Medicine Finland).

The samples of standard- and low-risk patients will be collected and stored in national biobanks of each country for later research. This will include comparison of MFC-MRD negativity with molecular MRD analyses and, depending on funding, comparison of result of genomic profile with respective of high-risk patients to find biomarkers and predictors for response.
1.6 Potential risks and benefits

Referring 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 Millennium. 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.

This study will be conducted in compliance with the protocol, good clinical practice (GCP), applicable regulatory requirements, and International Conference on Harmonisation (ICH) guidelines.

Ixazomib plus lenalidomide and dexamethasone has been compared with lenalidomide and dexamethasone in phase 3 study (Tourmaline-MM1) and there was no significant difference between reported grade 3 SAEs of these study arms.¹⁷

Oral safe and effective treatment would benefit patients and health care.

The potential benefits of participating in the study

Myeloma is according to current views, a chronic, incurable cancer disease. The induction treatment of myeloma, combined with high-dose chemotherapy and stem cell transplantation reduces the amount of cancer cells and is the standard treatment in myeloma. The standard induction treatment would include also outside trial proteasome inhibitor (subcutaneous bortezomib 4 injections per cycle), immunomodulating drug and dexamethasone. Post-transplant consolidation treatment is already shown to improve the response, but is not yet indicated in routine use. In studies performed outside Nordic countries, lenalidomide maintenance has already shown a clear benefit in the absence of the disease when compared with placebo, however, slightly worse for patients with high-risk chromosomal findings, who in this study will be treated with lenalidomide in combination with ixazomib. Induction treatment and the additional treatment after stem cell transplantation will be administered with oral capsules.

Possible disadvantages and inconveniences of the trial

Compared with normal treatment the patients with complete response in this trial may have
some additional bone marrow sample examinations, 1-2 per year, compared with patients outside study. The number of hospital visits is not higher compared with normal treatment visits. Unfortunately, all drugs used for treatment of myeloma can have side effects. Treatment of myeloma impairs the immune system; the patient might become more vulnerable to infections. The oral proteasome inhibitor ixazomib citrate has been shown to induce considerably less neuropathy than bortezomib which has been used earlier in this setting. Investigational drugs can cause side effects that are not known in advance, and because of this, the treatment should be carefully monitored.
2. STUDY OBJECTIVES

2.1 Primary Objectives

Primary objective is to investigate the protocol treatment efficacy based on serological and bone marrow analyses including minimal residual disease assessment by MFC and safety of IRd induction followed by ASCT, IRd consolidation and IR or R maintenance.

2.2 Secondary Objectives

Secondary objectives are the proportion of patients achieving MFC-MRD negative status during protocol treatment, progression-free survival (PFS), time to next treatment (TTNT) and overall survival (OS) in patients treated by protocol treatment. Additional secondary objectives are improvement of responses during consolidation, during risk stratified maintenance and evaluation whether high-risk patients will reach similar PFS, TTNT and OS than standard- and low-risk patients with this escalated therapy. Secondary objectives are also quality of life and health economy analyses.

2.3 Tertiary/Exploratory Objectives

Explorative objective is the genomic profiling of myeloma of high-risk patients in addition to drug sensitivity and resistance testing. This is a separative study funded by different grant from Celgene. The additional second explorative objectives, depending on sufficient funding, are the comparison of MFC-MRD negativity with molecular MRD and the comparison of genomic profile of SR-LR patients with HR patients to find out predictive biomarkers for response.

3. STUDY ENDPOINTS

3.1 PRIMARY ENDPOINTS

The primary endpoint is proportion of patients with minimal residual disease < 0.01% assessed by 8-color multiparameter flow cytometry at any time during study protocol treatment.

3.2 Secondary Endpoints

The first secondary endpoint is proportion of patients with negative minimal residual disease assessed by 8-color multiparameter flow cytometry at any time during protocol treatment. This will be assessed when last patient has been one year on maintenance.

Additional secondary endpoints:
Hospital District of Helsinki and Uusimaa/Helsinki University Hospital (HUS)
NMSG 23/15

- Safety
- Overall response rate (ORR)
- Progression-free survival (PFS) in both groups
- Improvement of responses and MRD negativity during maintenance in both groups
- Time to next treatment
- Quality of life
- Overall survival (OS) with 10 year follow up
- Type of treatment after relapse

3.3 Tertiary/Exploratory Endpoints
See page 35 Exploratory objectives.

4. STUDY DESIGN

4.1 Overview of Study Design

This is an investigator initiated, academic, non-randomized, open-label multicenter phase 2 study for newly diagnosed multiple myeloma patients in need of therapy and eligible for high-dose therapy supported by ASCT. Details of all treatments, scheduling and doses are given in section 7.

All patients will receive 4 cycles of ixazomib plus lenalidomide and dexamethasone (IRd) as induction followed by autologous cell stem cell mobilization and collection and single ASCT. Thereafter all patients will have 2 cycles of consolidation with IRd. Based on cytogenetic FISH findings at diagnosis patients will stratify into two different groups for maintenance, one for high-risk patients and one for standard-low-risk patients.

The primary objective is to assess the proportion of patients achieving the MFC-MRD < 0.01% during protocol treatment. This is used for sample size calculation. The hypothesis is that by this new treatment protocol 58% of patients will reach this level compared to the historical treatment where the respective level is in 45% of patients.

Study procedures:

At registration each patient will have an unique identification number and following details will be documented:

- Name of study center and responsible investigator
- Date of birth of study patient
- Date of signed informed consent
- Date of treatment start

- At entry: before the start of protocol treatment blood- and urine values within 2 weeks before study start, bone marrow samples within 4 weeks and bone imaging within 2 months
- During induction: within 7 days before next cycle
- Within 7 days before mobilization (optional: before HDT and ASCT)
- Within 14 days before consolidation
- Within 7 days before maintenance
- During maintenance after each maintenance cycle during first year, thereafter every 8 weeks
- At progression and if patient is taken/withdrawn off protocol

All patients will be followed until 10 years after registration

Side studies:

The samples of blood, bone marrow and skin (at dg) will be biobanked in national biobanks before treatment and at progression/relapse. In Finland the samples will be sent to the Institute for Molecular Medicine Finland where the samples of high-risk patients will be sequenced and tested for drug resistance and sensitivity. The aim is to find biomarkers for response prediction.

In addition to MFC-MRD negativity assessment molecular MRD will be compared with MFC-MRD.

Peripheral blood samples of nCR/CR will be sent with different finance in Sweden for NK- and T-cell analyses.

In Trondheim Norway detection of circulating tumor DNA in sequential samples at time point of MRD assessment is considered by Prof. Anders Waage.

4.2 Number of Patients

Total number of patients to be enrolled is 120 (sample size). The study patient is considered enrolled on first day of ixazomib plus lenalidomide and dexamethasone treatment. Any study related investigations are not started before written informed consent.
4.3 Duration of Study

Expected duration of treatment:

- screening 14 days
- induction 4-5 months
- stem cell mobilization, collection and ASCT with post-transplant supportive care 2 months
- consolidation 2 months, will be started within 100 days after HDT
- maintenance will be started within one month after consolidation until progression or toxicity

The first analyses of the study will be done for response evaluation when the last patient has been two years on maintenance treatment. The treatment of each individual patient will continue until progression or excess toxicity.

All patients will be followed until 10 years after registration.

5. STUDY POPULATION

5.1 Inclusion Criteria

Each patient must meet all of the following inclusion criteria to be enrolled in the study:

1. Newly diagnosed transplant eligible male or female multiple myeloma patients, 18-70 years of age, who have not received prior treatment for multiple myeloma

2. Symptomatic and measurable disease diagnosed by standard criteria (International Myeloma Working Group, CRAB criteria)

3. Voluntary written informed consent must be given before performance of any study related procedure not part of standard medical care, with the understanding that consent may be withdrawn by the patient at any time without prejudice to future medical care.

4. Female patients who:
   - Are postmenopausal for at least 1 year before the screening visit, OR
   - Are surgically sterile, OR
   - If they are of childbearing potential, fertile, agree to practice 2 effective methods of contraception (see 7.8 Pregnancy; acceptable contraception methods), at the same time, and agree to ongoing pregnancy testing and adhere to the guidelines of the lenalidomide pregnancy prevention program from the time of signing 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, post-ovulation methods] and withdrawal are not acceptable methods of contraception.)

Male patients, even if surgically sterilized (ie, status post-vasectomy), must agree to one of the following:

• Agree to practice effective barrier contraception and adhere to the guidelines of the lenalidomide pregnancy prevention program 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, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

4. Patients must have a diagnosis of a symptomatic multiple myeloma without any previous therapies except dexamethasone 160 mg dose, or comparable dose of other steroids, and local radiotherapy for symptom control

5. Eastern Cooperative Oncology Group (ECOG) performance status and/or other performance status 0, 1, or 2.

6. Patients must meet the following clinical laboratory criteria:

• Absolute neutrophil count (ANC) ≥ 1,000/mm$^3$ (≥ 1.0 x 10$^9$/L) and platelet count ≥ 75,000/mm$^3$ (75 x 10$^9$/L). Platelet transfusions to help patients meet eligibility criteria are not allowed within 3 days before study enrollment.

• Total bilirubin ≤ 1.5 × the upper limit of the normal range (ULN).

• Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 3 × ULN.

• Calculated creatinine clearance ≥ 30 mL/min (Cockcroft-Gault estimation of creatinine clearance (CRcl): CRcl (mL/min) = (140 - age) (weight [kg]) / 72 (serum creatinine [mg/dL]); for females, multiply by 0.85 (Cockcroft DW. 1976, Luke DR. 1990).

7. Patient must be willing and able to adhere to the study protocol visit schedule and other protocol requirements.

8. Negative pregnancy test at inclusion if applicable
5.2 Exclusion Criteria

Patients meeting any of the following exclusion criteria are not to be enrolled in the study:

1. Female patients who are lactating or have a positive serum pregnancy test during the screening period.

2. Major surgery within 14 days before enrollment.

3. Radiotherapy within 14 days before enrollment.

4. Central nervous system involvement with multiple myeloma.

5. Infection requiring systemic antibiotic therapy or other serious infection within 14 days before study enrollment.

6. Inability, unwillingness or contraindication to use thrombosis prophylaxis or antithrombotic therapy or herpes zoster prophylaxis.

7. Evidence of current uncontrolled cardiovascular conditions, including uncontrolled hypertension (blood pressure without medication $\geq 200/120$), uncontrolled cardiac arrhythmias (other than fibrillatio atriorum with adequate anticoagulation or supraventricular or ventricular extrasystolia, symptomatic congestive heart failure (NYHA classification Appendix 14.7), unstable angina, or myocardial infarction within the past 6 months.

8. Systemic treatment, within 14 days before the first dose of ixazomib, strong CYP3A inducers (rifampin, rifapentine, rifabutin, carbamazepine, phenytoin, phenobarbital), or use of Ginkgo biloba or St. John’s wort.

9. Ongoing or active systemic infection, active hepatitis B or C virus infection, or known human immunodeficiency virus (HIV) positive.

10. Any serious medical or psychiatric illness that could, in the investigator’s opinion, potentially interfere with the completion of treatment according to this protocol.

11. Known allergy to any of the study medications, their analogues, or excipients in the various formulations of any agent.

12. Known GI disease or GI procedure that could interfere with the oral absorption or tolerance of ixazomib or lenalidomide including difficulty swallowing.
13. Diagnosed or treated for another malignancy within 5 years before study enrollment or previously diagnosed with another malignancy and have any evidence of residual disease. Patients with nonmelanoma skin cancer or carcinoma in situ of any type are not excluded if they have undergone complete resection.

14. Patient has Grade 1 polyneuropathy with pain on clinical examination during the screening period.

15. 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.

16. Patients that have previously been treated for multiple myeloma or smoldering myeloma with ixazomib or any other therapy, or participated in a study with ixazomib whether treated with ixazomib or not.

17. Primary plasma cell leukemia, POEMS syndrome, Waldenström disease, myelodysplastic syndrome or myeloproliferative disease

18. Systemic AL amyloidosis/primary amyloidosis or myeloma associated amyloidosis.

19. Allogeneic stem cell transplantation planned

20. Participants receiving any other investigational agents or received within 60 days

6. STUDY INVESTIGATIONS

6.1 Medical previous history

• complete medical history
• symptoms of illness
• history of thrombosis of the patient and the relatives
• history of any other malignancies of the patient and relatives
• performance status (ECOG)
• infections
• bone symptoms
• bleeding
• polyneuropathy
• gastrointestinal symptoms
6.2 Physical examinations

- standard physical examination with cardiovascular and neurological (polyneuropathy, autonomic neuropathy) examinations
- orthostatic hypotension
- body weight and height, surface area
- exclusion of infections and bleedings

6.3 Hematology

- hemoglobin, hematocrit, leukocytes, leukocyte differential count, neutrophils, platelets

6.4 Blood chemistry

- creatinine, creatinine clearance (Cockcroft-Gault equation)
- liver enzymes (ALAT, ASAT, AFOS, BIL)
- plasma albumin
- serum beta-2-microglobulin
- LD
- CRP
- Ionized calcium, sodium, potassium, phosphate
- Uric acid
- T4v, TSH (thyreoida function)

Blood, bone marrow and urine samples will be required at diagnosis and at key time-points in follow-up for biochemical, and immunophenotypic assessments, as part of scientific studies in order to monitor MRD as defined by paraprotein, serum free light chain and flow cytometry to define depth and quality of responses. In addition, cytogenetic FISH findings are utilized as prognostic and stratification markers. Samples for later molecular assessment will be collected.

6.5 Immunochemistry

- Serum- and 24h urine- protein electrophoresis and immunofixation (IFE)
- For qualification of M-protein at entry and to confirm CR
- Quantification of serum- or urine M-component always when response is assessed
- Serum free light chain (sFLC) assay always when response is assessed
6.6 Bone marrow assessment

Bone marrow samples before treatment, if MRD-negative and at relapse for biobanking in all participating countries. Bone marrow aspiration at entry, after induction and consolidation and every 6 months during maintenance if \( \geq \) nCR/CR including morphology and immunophenotyping.

Bone marrow samples at diagnosis for FISH analysis (if IgH break apart positive), screening of translocations 4;14, 14;16 and 14;20. If abnormal IgH is found without these aberrations then translocations 11;14, 6;14, 8;14 (optional) should be analysed.

- del17p/monosomy
- del13q/13-
- 1q21 gain, 1p36 loss (del1p)

G-banding (hypodiploidy, hyperdiploidy, del13q/monosomy).
FISH analyses will be performed according to EMN guidelines (Ross et al. Haematologica 2012; 97:1272-1277). 

FISH analysis will be done in the cytogenetic laboratories of university hospitals, who are specialized to do myeloma-FISH panels. Most important and mandatory for analysis is to perform CD138+ cell purification. Thereafter the number of aberrant cells is so good that possible small variations no dot have influence on the result. IGH translocations appear typically in more than 10%, which is here the threshold for positive finding regarding translocations. Deletions can be the most difficult to interpret but the only important deletion to recognize in this study is del 17p, and for del 17p the threshold is 60%. Analysis are usually done by one very experienced geneticist but if the result is close to the threshold another geneticist will confirm the analysis. The percentage of all FISH findings will be reported for all study patients. Laboratories use CE- certified probes and participate regularly in quality control trials.

6.7 Specific additional investigations

- Bone imaging: Low-dose whole body CT or x-ray according to local MM protocols
  - at entry and before maintenance of affected bones
- Cardiological investigations, echo, if clinically needed
- Spirometry and diffusion capacity, if clinically needed
- MRI, PETCT (not routinely, performed for clinical reasons and in extramedullary disease)
Periferal blood for the T-, B-, myeloid and NK-cells phenotype analyses will be collected in parallel with MRD assessment.

Peripheral blood samples for circulating DNA will be planned to collect at the same time points as immunochemistry to prof. Anders Waage Trondheim, Norway. These samples will provide information about the level of specified mutated clones and this can be compared with the M-protein and MRD.
6.8 MINIMAL RESIDUAL DISEASE

6.8.1 Multiparameter flow cytometry

Before study treatment a diagnostic MFC for clonality assessment and patient specific panel design will be collected. Thereafter before mobilization, after ASCT, after consolidation and during the maintenance treatment every 6 months a bone marrow aspirate of nCR/CR patients will be taken for both morphology and MFC. Immunophenotyping is mandatory for patients in immunofixation negative CR with normal free light chain ratio to confirm stringent CR (sCR, no clonal plasma cells).

MRD will be measured by MFC according to recently published guidelines (Stetler-Stevenson M et al Cytometry B, 2015). In brief, nucleated cells of EDTA- anticoagulated bone marrow samples will be concentrated by prelysis of red cells (Bulk Lyse™, Cytognos) within 24 hours of collection. An aliquot containing $5 \times 10^6$ cells is stained for surface antigens with following eight-colour panel: CD38-FITC (multiepitope clone), CD56-PE (clone C5.9) and CD81 APC-C750 (clone M38) from Cytognos, Salamanca, Spain, CD117-APC (clone 104D2) and CD138-BV421 (clone MI15) from BD Biosciences, San Jose CA, CD45 PerCP Cy5.5 (clone HI30) from E Bioscience, CD19 PC7 (clone J3-119) from Beckman-Coulter and CD27-BV510 (clone O323) from Biolegend or corresponding MM-MRD kit (Cytognos, Spain Salamanca) with CD27 and CD138 as drop-in reagents (clones as above with the exception of CD19-PECy7 clone 19-1). Staining of intracytoplasmic kappa and lambda light chains with polyclonal reagents after permeabilization with Fix&Perm is optional. Specimens are acquired using BD FACSCanto II or Facs Systems (BD Biosciences, San Jose, CA) or Navios (Beckman-Coulter) flow cytometers. The instrument settings are harmonized with specified lots of rainbow beads according to Euroflow guidelines. Data is analyzed with Diva or Infinicyt soft wares. Plasma cells are gated with CD38 and CD138 expressions and abnormal plasma cells are distinguished from normal plasma cells according to previous recommendations (Rawstron AC et al Haematologica 2008; 93:431-438, Flores-Montero J et al Cytometry B, 2015, Arroz et al Cytometry B, 2015). MRD is calculated as percentage of total nucleated cells and of total plasma cells. A population of 50 events and 20 events are the lower limits for quantitation and detection, respectively. If MRD is not detected and the number of cells acquired is below $1 \times 10^6$ or if the bone marrow sample is not inadequate (no immature myeloid or lymphoid precursor, mast cells or normal plasma cells), the sample will be regarded as not representative. The multiparameter flow cytometry procedure is standardized in every step; settings of EuroFlow in flow cytometry devices, standard operating procedures of EuroFlow for bulk lysis
and multiple myeloma-minimal residual disease panel. In order to ensure the consistency of analyses quality control surveys of electronic files will be regularly performed.

6.9 Translational part of study

Samples for biobanking will be collected at multiple time points (prior to treatment and at relapse). Bone marrow MNCs will be viably cryopreserved for later translational study purposes including molecular profiling (exome and RNA sequencing) and immunophenotyping. As a separate research project CD138+ cells from high-risk patients will be tested in a drug sensitivity and resistance testing (DSRT) assay, which is a functional test to assess the ex-vivo sensitivity of the cells against the panel of approximately 150 small molecule inhibitors including multiple myeloma drugs. In addition, exome sequencing will be done for the collected high-risk patient samples. These assays will be done as part of a separately funded and designed project (Celgene/CITRE/FIMM).

The samples of standard- and low-risk patients will be collected and stored in national biobanks of each country for later research. Depending on funding, this will include a molecular MRD assay and comparison of results of genomic and transcriptomic profiles of high-risk patients to find biomarkers and predictors for response.

The MRD- method of the study is multiparameter flow cytometry, but molecular analysis of MFC-MRD-negative patients will be performed (depending on sufficient funding) to compare the 8-color Euroflow assay sensitivity with molecular sensitivity.

Patients who are MRD negative by MFC are analysed using the Adaptive Biotechnologies NGS-MRD Clonoseq platform and that can be coordinated by FIMM (Institute for Molecular Medicine Finland).

Samples for translational research:

For DNA based MRD assessment
1) Exome sequencing: 20 ml bone marrow to Ficoll and CD138+ selection frozen cells or extracted DNA shipped
Skin biopsy: frozen tissue or extracted DNA shipped

2) Molecular assay (NGS) IgH, K/L: 10 ml bone marrow
CD138+ selection – frozen cells – DNA extraction
7. STUDY DRUGS

7.1. Description of investigational agent ixazomib

Ixazomib Capsules
The ixazomib drug product is provided in strengths of 4.0-, 3.0-, and 2.3-mg and 2.0-, 0.5-, and 0.2 mg capsules as the active boronic acid. The different dose strengths are differentiated by both capsule size and color as described below:

<table>
<thead>
<tr>
<th>Dose Strength</th>
<th>Capsule Size</th>
<th>Capsule Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0 mg</td>
<td>Size 4</td>
<td>Ivory</td>
</tr>
<tr>
<td>3.0 mg</td>
<td>Size 3</td>
<td>Light gray</td>
</tr>
<tr>
<td>2.3 mg</td>
<td>Size 2</td>
<td>Light pink</td>
</tr>
<tr>
<td>2.0 mg</td>
<td>Size 2</td>
<td>Swedish orange</td>
</tr>
<tr>
<td>0.5 mg</td>
<td>Size 3</td>
<td>Dark green</td>
</tr>
<tr>
<td>0.2 mg</td>
<td>Size 4</td>
<td>White opaque</td>
</tr>
</tbody>
</table>

For additional details, please see the ixazomib IB.

7.2 Study drug administration

7.2.1 Induction

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose/day</th>
<th>Route</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ixazomib</td>
<td>4 mg</td>
<td>Oral</td>
<td>1, 8, 15</td>
</tr>
<tr>
<td>Lenalidomide</td>
<td>25 mg</td>
<td>Oral</td>
<td>1-21</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>40 mg</td>
<td>Oral</td>
<td>1, 8, 15, 22</td>
</tr>
</tbody>
</table>

7.2.2 STEM CELL MOBILIZATION AND COLLECTION (ROUTINE PART OF STUDY)

7.2.2.1 Stem cell mobilization

This part is routine treatment of the patients and will be performed according to local procedures at each site. Possible mobilization regimens are cyclophosphamide + G-CSF or G-CSF alone, plerixafor if needed.
7.2.2.2. Stem cell collection:

The procedure will be performed according to the local standard protocols. If insufficient number of stem cells is collected a new mobilization attempt will be considered with G-CSF plus plerixafor.

7.2.2.3. High dose melphalan and stem cell transplantation

High dose melphalan and stem cell transplantation will be scheduled in 3-4 weeks after stem cell collection.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose/day</th>
<th>Route</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melphalan</td>
<td>200mg/m²</td>
<td>Iv</td>
<td>-2</td>
</tr>
<tr>
<td>Stem cell infusion</td>
<td>At least 2 x 10⁶/kg</td>
<td>Iv</td>
<td>0</td>
</tr>
<tr>
<td>Filgrastim (optional)</td>
<td>5 µg/kg</td>
<td>Sc</td>
<td>optional</td>
</tr>
</tbody>
</table>

In case of renal failure the dose of melphalan will be reduced to 140 mg/m². The manufacturer recommends that initial doses of melphalan should be reduced by 50% if the GFR is 40-50ml/min.

Stem cell transplantation procedure will be performed according to the local standard protocols. Tandem transplant is not included in the protocol. Centers which have policy to collect two autologous grafts will store second graft for progression.

Consolidation treatment will be started within 100 days after ASCT.

- ANC must be ≥ 1,000/mm³ = 1 x 10⁹/l
- Platelet count must be ≥ 75,000/mm³ = 75 x 10⁹/l
- All other nonhematologic toxicity (except for alopecia) must have resolved to ≤ Grade 1 or to the patient’s baseline condition

7.2.3 Consolidation

<table>
<thead>
<tr>
<th>2 consolidation cycles by this schedule of each 28-day cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agent</td>
</tr>
<tr>
<td>Ixazomib</td>
</tr>
<tr>
<td>Lenalidomide</td>
</tr>
<tr>
<td>Dexamethasone</td>
</tr>
</tbody>
</table>
7.2.4  Maintenance

Maintenance treatment will be started within one month after consolidation.

- ANC must be $\geq 1,000/mm^3 = 1 \times 10^9/l$
- Platelet count must be $\geq 75,000/mm^3 = 75 \times 10^9/l$
- All other nonhematologic toxicity (except for alopecia) must have resolved to $\leq$ Grade 1 or to the patient’s baseline condition

<table>
<thead>
<tr>
<th>High-risk group: Ixazomib plus lenalidomide maintenance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agent</strong></td>
</tr>
<tr>
<td>Ixazomib</td>
</tr>
<tr>
<td>Lenalidomide</td>
</tr>
<tr>
<td>Lenalidomide dose will be escalated after three first cycles to:</td>
</tr>
<tr>
<td>Lenalidomide</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Standard- and low-risk groups: Lenalidomide alone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agent</strong></td>
</tr>
<tr>
<td>Lenalidomide</td>
</tr>
<tr>
<td>Lenalidomide dose will be escalated after three first cycles to:</td>
</tr>
<tr>
<td>Lenalidomide</td>
</tr>
</tbody>
</table>

7.2.5  IXAZOMIB

Induction: Ixazomib 4 mg will be taken orally on days 1, 8, 15 of each 28-day cycles, four cycles together. The patient will receive the dose for each individual cycle for at-home use on the study visit before each cycle.

Consolidation: Consolidation treatment will be started within 100 days after ASCT. Ixazomib 4 mg will be taken orally on days 1, 8, 15 of each 28-day cycles, two cycles together. The patient will receive the dose for each individual cycle for at-home use on the study visit before each cycle.

Maintenance: Ixazomib 4 mg will be taken orally on days 1, 8 and 15 of each 28-day cycles in high-risk patient group. Maintenance will start within one month after consolidation. The patient will receive the dose for each individual cycle for at-home use on the study visit every 1 to 3 months depending on study course. Maintenance treatment will continue until disease progression or unacceptable toxicity.
Maintenance lenalidomide: Maintenance will start within one month after consolidation. Lenalidomide 10 mg will be started orally on days 1-21 of a 28-day cycles in all patient groups. In all risk groups the dose will be escalated to 15 mg on days 1-21 of a 28-day cycle. The patient will receive the dose for each individual cycle for at-home use on the study visit every 1 to 3 months depending on study course. Maintenance treatment will continue until disease progression or unacceptable toxicity.

All protocol-specific criteria for administration of the study drug combination (lenalidomide, ixazomib and dexamethasone during induction and consolidation, or the combination of lenalidomide and ixazomib OR lenalidomide only during maintenance) must be met and documented before drug administration. Study drugs 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, lenalidomide and dexamethasone should be modified as needed to accommodate patient tolerance to treatment; this may include symptomatic treatment, dose interruptions, and adjustments of these drugs (see Section 7.3).

Ixazomib Administration

Ixazomib will be supplied by Millennium as capsules of 2.3-, 3.0- and 4.0 mg ixazomib.

The prescribed administration of ixazomib doses in this study is 4 mg ixazomib in a 28-day cycle.

Ixazomib should be taken once a week on the same day and at approximately the same time for the first three weeks of a four week 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 in the morning on an empty stomach (no food or drink) at least 1 hour before or at least 2 hours after food. Each capsule should be swallowed separately with a sip of water. A total of approximately 8 ounces (240 mL) of water should be taken with the capsules.

Missed doses can be taken as soon as the patient remembers if the next scheduled dose is 72 hours or more away. A missed dose should not be taken within 72 hours of the next scheduled dose. 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. The patient should resume dosing at the time of the next scheduled dose.

Patients will fill drug medication diary during protocol treatment to document their doses.
Ixazomib Destruction: Investigational ixazomib (expired or end of study) should be destroyed on site according to the institution’s standard operating procedure. Be sure to document removal and destruction on drug accountability logs.

7.2.6 LENALIDOMIDE

Induction: Lenalidomide 25 mg will be taken orally on days 1-21 of each 28-day cycles, four cycles together. The patient will receive the dose for each individual cycle for at-home use on the study visit before each cycle.

Consolidation: Consolidation treatment will be started within 100 days after ASCT. Lenalidomide 25 mg will be taken orally on days 1-21 of each 28-day cycles, two cycles together. The patient will receive the dose for each individual cycle for at-home use on the study visit before each cycle.

Maintenance: Maintenance will start within one month after consolidation. Lenalidomide 10 mg will be started orally on days 1-21 of a 28-day cycles in all patient groups. In all risk groups the dose will be escalated after three first cycles to 15 mg on days 1-21 of a 28-day cycle. The patient will receive the dose for each individual cycle for at-home use on the study visit every 1 to 3 months depending on study course. Maintenance treatment will continue until disease progression or unaccepted toxicity.

Lenalidomide should be taken approximately at the same time on each treatment day with a small amount of water. If lenalidomide dose is delayed or missed, the dose should be taken only if no more than 12 hours have spent from missed dose. If more than 12 hours have spent, this dose will be omitted. A double dose should not be taken.

Patients will fill drug medication diary during protocol treatment to document their doses.

7.2.7 DEXAMETHASONE Induction: Dexamethasone 40 mg will be taken orally on days 1, 8, 15 and 22 of each 28-day cycles, four cycles together. The patient will receive the drug from public pharmacy by the national reimbursement principles or by local routines of each country for at-home use (see 1.4.). The dose of dexamethasone will be confirmed on each visit to patient.

Consolidation: Consolidation treatment will be started within 100 days after ASCT. Dexamethasone 40 mg will be taken orally on days 1, 8, 15 and 22 of each 28-day cycles, two cycles together. The patient will receive the drug from public pharmacy by the national
reimbursement principles of each country for at-home use. The dose of dexamethasone will be confirmed on each visit to patient.

Maintenance: There will be not any dexamethasone doses during maintenance.

Dexamethasone 40 mg will be taken orally with the morning breakfast. This drug will be reimbursed as routine medication. Patients will fill drug medication diary during protocol treatment to document their doses.

7.3 Dose-Modification Guidelines

Study drug dose modifications may change depending on the disease and combination with other drug(s). If a new combination study, an attempt should be made to integrate dose modifications for the combination for each AE.

7.3.1 Recommended Criteria for Beginning or Delaying a Subsequent Treatment Cycle & Dose Modifications for Treatment Associated Toxicity

Treatment will use a cycle length of 28 days. For a new cycle of treatment to begin, the patient must meet the following criteria:

- ANC must be ≥ 1,000/mm³ (1.0 x 10⁹/L)
- Platelet count must be ≥ 75,000/mm³ (75 x 10⁹/L)
- All other nonhematologic toxicity (except for alopecia) must have resolved to ≤ Grade 1 or to 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 will be 3 weeks or at the discretion of the Principal Investigator.

For dosing recommendations upon recovery, refer to Table 7.3.2 this page
Table 6-1 Ixazomib Dose Adjustments

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Dose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting Dose</td>
<td>4.0 mg</td>
</tr>
<tr>
<td>-1</td>
<td>3.0 mg</td>
</tr>
<tr>
<td>-2</td>
<td>2.3 mg</td>
</tr>
<tr>
<td>-3</td>
<td>Discontinue</td>
</tr>
</tbody>
</table>

Patients with calculated creatinine clearance (CrCl) < 30 ml/min will not be included in this study, but the dose reduction of ixazomib to 3 mg should be followed if the CrCl falls below < 30 ml/min.

Table 7.3.2  Recommended Dose Modifications for Ixazomib in Combination with Lenalidomide and Dexamethasone Treatment Associated Toxicity

Initiation of each cycle and maintenance will be started if neutrophil count is ≥ 1.0 x 10⁹/l and platelet > 75 x 10⁹/l. These guidelines are for dose modification during a cycle.

<table>
<thead>
<tr>
<th>Hematological Toxicities</th>
<th>Recommended Actions</th>
</tr>
</thead>
</table>
| **Thrombocytopenia (Platelet Count):** Platelet count less than 30 000/mm³ = 30 x 10⁹/l | • Withhold ixazomib and lenalidomide until platelet count is at least 30 x 10⁹/l.  
  • Following recovery, resume lenalidomide at the next lower dose according to its prescribing information and resume ixazomib at its most recent dose.  
  • If platelet count falls to less than 30 x 10⁹/l again, withhold ixazomib and lenalidomide until platelet count is at least 30 x 10⁹/l.  
  • Following recovery, resume ixazomib at the next lower dose and resume lenalidomide at its most recent dose. |

| Neutropenia (Absolute Neutrophil Count) | Absolute neutrophil count less than 500/mm³ = 0.5 x 10⁹/l. | • Withhold ixazomib and lenalidomide until absolute neutrophil count is at least 500/mm³ = 0.5 x 10⁹/l.  
  • Following recovery, resume lenalidomide at the next lower dose according to its prescribing information and resume ixazomib at its most recent dose.  
  • If absolute neutrophil count falls to less than 0.5 x 10⁹/l again, withhold ixazomib and lenalidomide until absolute neutrophil count is at least 0.5 x 10⁹/l.  
  • Following recovery, resume ixazomib at the next lower dose and resume lenalidomide at its most recent dose.  
  • For additional occurrences, alternate dose modification of lenalidomide and ixazomib. |
**Hematological Toxicities**

<table>
<thead>
<tr>
<th>Recommended Actions</th>
<th>Grade 3 nonhematologic toxicity judged to be related to lenalidomide and/or ixazomib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hold lenalidomide and ixazomib until resolution to Grade &lt; 1 or baseline</td>
<td>If not recovered to &lt; Grade 1 or baseline within 4 weeks</td>
</tr>
<tr>
<td>Reduce either lenalidomide or ixazomib depending which drug contributed to the toxicity (investigator judgment) to next lower dose upon return to &lt; Grade 1 or baseline</td>
<td>Subsequent recurrence of the same toxicity Grade 3 that does not recover to &lt; Grade 1 or baseline within 4 weeks</td>
</tr>
<tr>
<td>Hold lenalidomide and ixazomib until resolution to Grade &lt; 1 or baseline</td>
<td>For overlapping toxicity reduce the drug that was not reduced in the first instance to next lower dose, otherwise reduce the drug which contributed to toxicity (investigator judgment)</td>
</tr>
</tbody>
</table>

**Non-Hematological Toxicities**

<table>
<thead>
<tr>
<th>Recommended Actions</th>
<th>Rash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 2 or 3 rash not limiting activities of daily life symptomatic treatment with careful follow-up</td>
<td>Grade 2 or 3 (CTCAE 4.03)</td>
</tr>
<tr>
<td>Grade 3 rash discontinue temporarily lenalidomide and ixazomib until rash recovers to ≤ grade 1</td>
<td>• Grade 4 rash</td>
</tr>
<tr>
<td>Following recovery, resume lenalidomide at the next lower dose according to its prescribing information and continue ixazomib with previous dose</td>
<td>Discontinue whole treatment regimen permanently.</td>
</tr>
<tr>
<td>If grade 2 or 3 rash occurs again, withhold ixazomib and lenalidomide until rash recovers to grade 1 or lower.</td>
<td>For additional occurrences, alternate dose modification of lenalidomide and ixazomib</td>
</tr>
<tr>
<td>Following recovery, resume ixazomib at the next lower dose and resume lenalidomide at its most recent dose.</td>
<td></td>
</tr>
<tr>
<td>For grade 4 rash, discontinue whole treatment regimen permanently.</td>
<td></td>
</tr>
</tbody>
</table>

**Peripheral Neuropathy**

<table>
<thead>
<tr>
<th>Recommended Actions</th>
<th>Grade 1 Peripheral neuropathy with pain or grade 2 peripheral neuropathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Withhold ixazomib until peripheral neuropathy recovers to grade 1 or lower without pain or patient’s baseline</td>
<td>Grade 1 Peripheral neuropathy with pain or grade 2 peripheral neuropathy</td>
</tr>
<tr>
<td>Continue lenalidomide and dexamethasone</td>
<td>• Withhold ixazomib. Lenalidomide will continue. Toxicities should, at the physician’s discretion, generally recover to patient’s baseline condition or grade 1 or lower prior to resuming ixazomib</td>
</tr>
<tr>
<td>Following recovery, resume ixazomib at its most recent dose.</td>
<td>• Following recovery, resume ixazomib at the next lower dose</td>
</tr>
<tr>
<td>Grade 2 Peripheral Neuropathy with pain or grade 3 peripheral neuropathy</td>
<td>• Withhold ixazomib until peripheral neuropathy recovers to grade 1 or lower without pain or patient’s baseline</td>
</tr>
<tr>
<td>Grade 4 Peripheral Neuropathy</td>
<td>• Discontinue whole treatment regimen permanently.</td>
</tr>
</tbody>
</table>
Other Non-Hematological Toxicities

Hypo/hyperthyroidism ≥ Grade 2:

Hold doses of ixazomib, lenalidomide and dexamethasone for remainder of cycle. Initiate appropriate medical therapy. Maintain dose levels of lenalidomide, ixazomib and dexamethasone when dosing restarts at next cycle at discretion or treating physician.

Other Grade 4 Non-Hematological Toxicities judged to be related to lenalidomide and/or ixazomib

- Consider permanently discontinuing study drug
- Exceptions are cases in which the investigator determines the patient is obtaining a clinical benefit
- After recovery to grade 1 or to patient’s baseline lenalidomide and ixazomib must be continued with next lower dose with extreme caution

Once ixazomib or lenalidomide is reduced for any toxicity, the dose may not be re-escalated. Study patient can continue study treatment with 2-drug regimen (induction, consolidation) or one-drug treatment (maintenance) if one of the study drugs has to be permanently discontinued.

7.3.3 Lenalidomide - Dose modification for lenalidomide

Starting dose adjustment for renal impairment: Since lenalidomide is primarily excreted unchanged by the kidney, adjustments to the starting dose of lenalidomide are recommended to provide appropriate drug exposure in patients with moderate or severe renal impairment and in patients on dialysis. Based on a pharmacokinetic study in patients with renal impairment due to nonmalignant conditions, lenalidomide starting dose adjustment is recommended for patients with CreaCl < 60 mL/min. Non-dialysis patients with CreaCl less than 11 mL/min, and dialysis patients with creatinine clearances less than 7 mL/min, have not been studied. The recommendations for initial starting doses for patients with multiple myeloma (MM) are as follows:

<table>
<thead>
<tr>
<th>Renal function (GFR)</th>
<th>Starting dose of lenalidomide (induction)</th>
<th>Starting dose of lenalidomide for maintenance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal renal function/ Mild renal impairment ≥ 60 ml/min</td>
<td>25 mg every 24 hours</td>
<td>10 mg or 15 mg every 24 hours</td>
</tr>
<tr>
<td>Moderate Renal Impairment (between 30 – 60 ml/min)</td>
<td>10 mg every 24 hours</td>
<td>5 mg every 24 hours</td>
</tr>
</tbody>
</table>
Patients with a calculated creatinine clearance < 30 ml/min will not be included in this study, but the following dose modifications for lenalidomide will apply if the patient develop renal impairment on study treatment.

<table>
<thead>
<tr>
<th>Severe Renal Impairment (&lt; 30 ml/min, not requiring dialysis)</th>
<th>15 mg every 48 hours</th>
<th>5 mg every 48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>End Stage Renal Disease (&lt; 30 ml/min, requiring dialysis)</td>
<td>5 mg once daily</td>
<td>On dialysis days the dose should be administered after dialysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 mg 3 times a week after each dialysis</td>
</tr>
</tbody>
</table>

If there were dose modifications or delays in the previous cycle, use the following guidelines:

- If the cycle was completed without requiring further dose modification, then the next cycle will start at the same reduced dose of lenalidomide.
- If lenalidomide was held during the previous cycle and restarted at a reduced dose level, without interruption for the remainder of the cycle, then the reduced dose level will be initiated on Day 1 of the new cycle.
- If lenalidomide dosing was omitted for the rest of the previous cycle or if a new cycle is delayed due to lenalidomide-related toxicity newly encountered on the scheduled Day 1, then the new cycle will be started with one-level dose reduction.

There are currently no recommendations for dose adjustment of lenalidomide in patients with hepatic insufficiency. By IB v 18: PK analyses included 16 patients with mild hepatic impairment (total bilirubin > 1 to ≤ 1.5 x the ULN or AST > ULN) showed that mild hepatic impairment does not influence the disposition of lenalidomide. No data are available for patients with moderate-to severe hepatic impairment. Patients should be carefully followed for hepatic function based on reported previous liver toxicity.

### 7.3.4 Maintenance treatment with lenalidomide and ixazomib or with lenalidomide alone

Lenalidomide and ixazomib combination or lenalidomide alone maintenance will be started within one month after consolidation with 28-day cycle in each group.

<table>
<thead>
<tr>
<th>High-risk group: Ixazomib plus lenalidomide maintenance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agent</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Ixazomib</td>
</tr>
<tr>
<td>Lenalidomide</td>
</tr>
</tbody>
</table>

Starting dose of lenalidomide | 1st dose reduction | 2nd dose reduction | 3rd dose reduction | 4th dose reduction | 5th dose reduction |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg x 1 po</td>
<td>20 mg x 1 po</td>
<td>15 mg x 1 po</td>
<td>10 mg x 1 po</td>
<td>5 mg x 1 po</td>
<td>Discontinue lenalidomide</td>
</tr>
<tr>
<td>On days 1-21 every 28 days</td>
<td>on days 1-21 every 28 days</td>
<td>on days 1-21 every 28 days</td>
<td>on days 1-21 every 28 days</td>
<td>on days 1-21 every 28 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Lenalidomide dose will be escalated after three first cycles to:

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose/day</th>
<th>Route</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lenalidomide</td>
<td>15 mg</td>
<td>Oral</td>
<td>1-21</td>
</tr>
</tbody>
</table>

Lenalidomide and ixazomib will be started if neutrophil count is $\geq 1.0 \times 10^9/l$ and platelet $> 75 \times 10^9/l$. Dose modification during a cycle will be done by the guidelines presented in Table 7.3.2.

### Standard- and low-risk groups: Lenalidomide alone

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose/day</th>
<th>Route</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lenalidomide</td>
<td>10 mg</td>
<td>Oral</td>
<td>1-21</td>
</tr>
</tbody>
</table>

Lenalidomide dose will be escalated after three first cycles to:

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose/day</th>
<th>Route</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lenalidomide</td>
<td>15 mg</td>
<td>Oral</td>
<td>1-21</td>
</tr>
</tbody>
</table>

Lenalidomide will be started if neutrophil count is $\geq 1.0 \times 10^9/l$ and platelet $> 75 \times 10^9/l$. Dose modification during a cycle will be done by the guideline presented in Table 7.3.2.

### Dose levels for lenalidomide alone during maintenance therapy

- Starting dose is 10 mg once daily on days 1-21 every 28 days
- Dose will be escalated to 15 mg after three first cycles
- Dose level -1 is 10 mg once daily on days 1-21 every 28 days
- Dose level -2 is 5 mg once daily on days 1-21 every 28 days
- Dose level -3 is no lenalidomide

### 7.3.5 Dose modification for lenalidomide alone for haematologic toxicity* during maintenance

#### Neutropenia

Neutrophil $< 0.5 \times 10^9/L =$ grade 4 neutropenia OR febrile neutropenia (fever $\geq 38.5^\circ C$ and neutrophil $< 1 \times 10^9/L$): Stop the dose for remainder of cycle.

If ANC is recovered / febrile neutropenia is resolved start next cycle. Decrease by 1 dose level when dosing restarts at next cycle.

#### Thrombocytopenia

Grade 4: Platelets $< 25 \times 10^9/L$

Stop the dose for remainder of cycle. If platelets are recovered start next cycle. Decrease by one dose level when dosing restarts at next cycle.

* Exclude other causes, especially progressive disease.
Dose modification instructions for lenalidomide alone for non-haematologic toxicity during maintenance

Rash = Grade 3: Hold dose for remainder of cycle. Decrease by one dose level when dosing restarted at next cycle (rash must resolve to ≤ Grade 1).
Rash = Grade 4 or blistering: Discontinue lenalidomide and discontinue subject from study

Constipation ≥ Grade 3: Hold dose for remainder of cycle. Initiate bowel regimen. Decrease by one dose level when dosing restarted at next cycle (Constipation must resolve to ≤ Grade 2)

Thrombosis/embolism ≥ Grade 3: Hold dose for remainder of cycle. Initiate anticoagulation treatment. Maintain dose level when dosing restarted at next cycle at discretion of treating physician.

Hypo/hyperthyroidism ≥ Grade 2: Hold dose for remainder of cycle. Initiate appropriate medical therapy. Maintain dose level when dosing restarted at next cycle at discretion of treating physician

Other non-hematological toxicity: Hold lenalidomide until toxicity resolves to ≤ grade 2 and contact study coordinator. After consultation with the study PI drug may be resumed at lower dose level, as described above.

Other non-hematological toxicity grade 4: Discontinue lenalidomide and contact study PI.

7.3.6 Dexamethasone- Dose modification for dexamethasone:

<table>
<thead>
<tr>
<th>Starting dose of dexamethasone</th>
<th>1st dose reduction</th>
<th>2nd dose reduction</th>
<th>3rd dose reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 mg/d on days 1, 8, 15 and 22</td>
<td>20 mg/d on days 1, 8, 15 and 22</td>
<td>10 mg/d on days 1, 8, 15 and 22</td>
<td>Discontinue dexamethasone</td>
</tr>
</tbody>
</table>

In case of dose reduction during initial IRd therapy, the participant will receive the reduced dose levels (the last level applied during initial therapy).
Thrombosis prophylaxis

There is an increased risk of thrombosis, predominantly deep venous thrombosis and pulmonary embolism (but also myocardial infarction and cerebrovascular events) in MM patients treated with lenalidomide and dexamethasone or other chemotherapy. Thrombosis prophylaxis with low molecular weight heparine (LMWH) is strongly recommended during induction and consolidation treatment. The decision of antithrombotic prophylaxis (asetsosalicylate acid or LMWH) during maintenance treatment needs to be considered by individual risk assessment based on prior thrombosis history, smoking, hypertension, hyperlipidemia and paraprotein status. At least ASA 100 mg per day will be given as prophylaxis for all patients. Study patients should be closely monitored for symptoms of thrombosis.

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’s 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

The following medicinal products and procedures are prohibited during the study.

- Excluded foods and dietary supplements include St. John’s wort and Ginkgo biloba
- Any antineoplastic treatment with activity against MM, other than study drugs
- Radiation therapy (note that, in general, the requirement for local radiation therapy indicates disease progression)
- Platelet transfusions to help patients meet eligibility criteria are not allowed within 3 days prior to study drug dosing for any dosing day
Permitted Concomitant Medications and Procedures

The following medications and procedures are permitted during the study:

- Antiemetics, including 5-HT3 serotonin receptor antagonists, may be used at the discretion of the investigator.

- Loperamide or other antidiarrheal should be used for symptomatic diarrhea at discretion of the investigator. The dose and regimen will be according to institutional guidelines. IVF should be given to prevent volume depletion.

- Growth factors (e.g., granulocyte colony stimulating factor [G-CSF], granulocyte macrophage-colony stimulating factor [GM-CSF], recombinant erythropoietin) are permitted. Their use should follow published guidelines and/or institutional practice. Erythropoietin will be allowed in this study. Their use should follow published guidelines and/or institutional practice.

- Patients should be transfused with red cells and platelets as clinically indicated and according to institutional guidelines.

- Antiviral prophylaxis and possible therapy such as acyclovir or valacyclovir will be administered (See chapter 7.9)

- Standard pneumocystis jirovecii pneumonia prophylaxis is recommended during dexamethasone treatment and after ASCT by the standards of each study site.

- Concomitant treatment with bisphosphonates will be permitted, as appropriate.

- Patients who experience worsening neuropathy from baseline may be observed for recovery, and have dose reductions/delays as indicated in the protocol, and any supportive therapy or intervention may be initiated as appropriate at the discretion of the investigator.

- Supportive measures consistent with optimal patient care may be given throughout the study.

Precautions and Restrictions

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

7.8 Pregnancy

It is not known what effects ixazomib has on human pregnancy or development of the embryo or fetus. For lenalidomide there is a specific Risk Minimization Plan for Clinical Trials to avoid embryo-fetal exposure, and this strategy is strongly mandatory to follow in the study. **Global Pregnancy Prevention Plan (PPP) Lenalidomide Adult is included as an Attachment in this protocol and it is used in EU.** Only highly effective methods of contraception are acceptable: The two methods of reliable contraception must include one highly effective method and one additional effective (barrier) method. The following are examples of highly effective and additional effective methods of contraception: Examples of highly effective methods: intrauterine device (IUD), hormonal (birth control pills, injections, implants, levonorgestrel-releasing intrauterine system (IUS), medroxyprogesterone acetate depot injections, ovulation inhibitory progesterone-only pills [e.g. desogestrel]), tubal ligation, partner’s vasectomy. Examples of additional effective methods: male condom, diaphragm, cervical cap. 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.)

Pregnancy testing is performed at the beginning of each 28-day cycle and at the end of study visit, applying to women of childbearing potential.

Lenalidomide and pregnancy

Females of childbearing potential: Pregnancies and suspected pregnancies (including elevated βhCG or positive pregnancy test in a female subject of childbearing potential regardless of age or disease state) occurring while the subject is on IP, or within 28 days of the subject´s last dose of IP, are considered immediately reportable events. IP is to be discontinued immediately and the subject instructed to return any unused portion of the IP to the Investigator. The female subject should be referred to an obstetrician-gynecologist, preferably one experienced in reproductive toxicity for further evaluation and counseling. The investigator will follow the subject until completion of the pregnancy, and must notify the Sponsor-Principal Investigator immediately about the outcome of the pregnancy (either normal or abnormal outcome). If the outcome of the pregnancy was abnormal (e.g., spontaneous or therapeutic abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE within 24 hours of the Investigator´’s knowledge of the event using the SAE Report Form. All neonatal deaths that occur within 28 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 28 days that the Investigator suspects is related to the in uteri exposure to the IP should also be reported as an SAE within 24 hours of the Investigator´’s knowledge of the event using the SAE Report Form, or approval equivalent form.

Male subjects: If a female partner of a male subject taking investigational product becomes pregnant, the male subject taking IP should notify the Investigator, and the pregnant female partner should be advised to call their healthcare provider immediately. If a pregnancy related event is reported in a female partnet of a male subject, the investigator should ask if the female
partner is willing to share information with Drug Safety and allow the pregnancy related event to be followed up to completion.

7.9 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.

Prophylaxis Against Risk of Reactivation of Herpes Infection

Patients may be at an increased risk of infection including reactivation of herpes zoster and herpes simplex viruses. Prophylactic antiviral therapy is required for every patient while receiving study treatment. Examples of acceptable antiviral therapy include acyclovir (eg, 400 mg given orally, 2 or 3 times a day), famcyclovir (eg, 125 mg given orally, twice a day), or valacyclovir (eg, 500 mg given orally, once or twice a day) according to local standard of site.

Nausea and/or Vomiting

Standard anti-emetics including 5-hydroxytryptamine 3 serotonin receptor antagonists are recommended for emesis if it occurs once treatment is initiated; prophylactic anti-emetics may also be considered at the physician’s discretion. Dexamethasone should not be administered as an anti-emetic. Fluid deficit should be corrected before initiation of study drug and during treatment.

Diarrhea

Prophylactic antidiarrheals will not be used in this protocol. However, 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 deficit should be corrected before initiation of treatment and during treatment.

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-threatening rash with skin peeling and mouth sores, toxic epidermal necrolysis, or drug reaction with eosinophilia and systemic symptoms (DRESS) which have been observed in combination treatment with ixazomib and which should be managed symptomatically according to standard medical practice including stopping treatment with ixazomib. Punch biopsies for histopathological analysis are encouraged at the discretion of the investigator.

**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 7.3.2). 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 7.3.2). Therapy can be reinitiated at a reduced level upon recovery of ANCs.

**Fluid Deficit**

Dehydration should be avoided since ixazomib may cause vomiting, diarrhea, and dehydration. Acute renal failure has been reported in patients treated with ixazomib, commonly in the setting of the previously noted gastrointestinal toxicities and dehydration.

Fluid deficit should be corrected before initiation of study drug and as needed during treatment to avoid dehydration.
Hypotension

Symptomatic hypotension and orthostatic hypotension with or without syncope have been reported with ixazomib. 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. Fluid deficit should be corrected before initiation of study drug and as needed during treatment to avoid dehydration.

Posterior Reversible Encephalopathy Syndrome

One case of posterior reversible encephalopathy syndrome, which ultimately resolved, has been reported with ixazomib. This condition is characterized by headache, seizures and visual loss, as well as abrupt increase in blood pressure. Diagnosis may be confirmed by magnetic resonance imaging (MRI). If the syndrome is diagnosed or suspected, symptom-directed treatment should be maintained until the condition is reversed by control of hypertension or other instigating factors.

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.

7.10 Preparation, Reconstitution, and Dispensing of Ixazomib

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

Details for lenalidomide see chapter 1.3

7.11 Packaging and Labeling of Ixazomib

The study drug ixazomib capsules will be provided by Millennium. The study drug will be labeled and handled as open-label material, and packaging labels will fulfill all requirements specified by governing regulations.

The capsules are individually packaged using foil-foil blisters that are in a child-resistant carton. There are 3 capsules in each wallet/carton.
7.12 Storage, Handling, and Accountability of Ixazomib

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 between +2°C and +30°C. Do not freeze ixazomib or store above 30°C. Ensure that the drug is used before the retest expiry date provided by Millennium. 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 refrigerated 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 between +2°C and +30°C. Do not freeze ixazomib or store above 30°C for the duration of each cycle. Patients should be instructed to return their empty blister packs to the investigative site, rather than discarding them. Reconciliation will occur accordingly when the patient returns for their next cycle of take-home medication. 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 cleanup 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.

Details for lenalidomide see chapter 1.3

7.13 Study Compliance

Study drug will be administered or dispensed only to eligible patients under the supervision of the investigator or identified sub-investigator(s). The appropriate study personnel will maintain records of study drug receipt and dispensing. The personnel will maintain accountability records of study drug, including information such as study drug name, dose/strength, batch number, date and no. of capsules dispensed, date and no. of capsules returned and finally date destroyed.

7.14 Treatment Assignment

Patients will have similar treatment during induction and consolidation. The maintenance treatment will be administered based on risk stratification by FISH analyses before treatment.

7.15 Termination of Treatment and/or Study Participation

Patients will be informed that they have the right to withdraw from the study at any time for any reason, without prejudice to their medical care. The investigator also has the right to withdraw patients from the study for any of the following reasons:

- Adverse event
- Protocol violation
- Lost to follow-up
- Progressive disease
- Study terminated
- If the responsible physician thinks a change of therapy would be best for the patient
- No compliance of the patient
- Pregnancy
- Death

Patients who are withdrawn from the study will not be replaced. At the time of withdrawal, all study procedures outlined for the End of Study visit should be completed. The primary reason for patient’s withdrawal from the study should be recorded in the source documents and CRF. If withdraw by investigator or patient will occur, the patient undergoes an end of study assessment, in which present disease stage and reason for end of study should be noted and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable.
8. RESPONSE ASSESSMENT

Responses will be assessed by the International Working Group Criteria, see Appendix 14.5

9. STATISTICAL AND QUANTITATIVE ANALYSES

9.1 Statistical Methods

Efficacy analyses will be performed in efficacy evaluable population or ITT population per specified. Patients who have been considered ineligible afterwards based on information that should have been available before registration and start of protocol treatment will be excluded from analyses. Kaplan-Meier method is used to estimate the time to event analyses. PFS and OS will be calculated from the start of the study protocol treatment. All p-values will be reported as 2-sided.

9.1.1 Determination of Sample Size

Statistical study plan: This is a phase II prospective study design where the sample size of 120 patients (see below) is required to show whether the proportion of patients achieving MRD < 0.01% assessed by multiparameter flow cytometry (MFC) could be improved from the estimated 45%, of the ongoing FMG-MM02 (NCT01790737) including RVD induction + ASCT + lenalidomide maintenance, to 58% in this study. The 58% is based on the study of Roussel et al.\(^5\) where the best MRD neg response by MFC was from 58% to 68% after RVD induction + ASCT + RVD consolidation + lenalidomide maintenance.

In FMG-MM02 the sensitivity of MFC-MRD negativity is < 0.01%, but we have included in our panel CD27 and CD117 antibodies, in contrast to Roussel et al. panel, where they have the sensitivity of 0.0025% without these antibodies. For this NMSG trial we will develop an updated MFC method based on recent recommendations of the group of leading experts on MFC-MRD field (Stetler-Stevenson et al. in references). With this new MFC assay the achievable sensitivity of MRD- negative sample could be < 0.001%. Therefore the negative results are not straight comparable, and we use the level MFC-MRD < 0.01% as the primary endpoint, and MFC-MRD- negativity as a secondary endpoint.

Ixazomib has shown to have improved pharmacokinetic and bioavailability compared to bortezomib so it is expected that the efficacy of IRd combination would be at least the same level (58%-68%) (Offidani et al.\(^5\)). Looking at the results of KRd upfront (Jakubowiak et al.) they received sCR rate of 61% with at least 8 cycles.\(^6\) With limited number of patients in the
study of Kumar et al., IRd for NDMM 22/65 patients went to ASCT and 7/7 CR patients and 8/9 VGPR patients were MRD-negative. MFC-MRD < 0.01% will be used as primary endpoint for the sample size calculation.
Efficacy:

Responses will be analyzed using efficacy evaluable population. Responses will be assessed:

- After 2 IRD cycles (serological responses)
- After 4 IRD cycles, before mobilization (serological, if nCR/CR: bone marrow, MFC-MRD, imaging if clinically needed)
- Before ASCT (serological)
- 3 months after ASCT (same assessment as at mobilization)
- After consolidation= before maintenance (same as at mobilization)
- Every 6 months during maintenance in each group (HR and SR/LR)
  - nCR/CR patients (serological, bone marrow, MFC-MRD)
  - molecular sample to stored if MFC-MRD negative
  - If response worse than CR (serological only)

Progression-free survival (PFS)

PFS is defined as time from start of study treatment to progression, relapse or death whichever comes first. Association of PFS with MRD negativity and positivity and clinical parameters at diagnosis will be analyzed. PFS will be analyzed using ITT population

Overall survival (OS)

OS will be defined as time from start of the treatment to death due to any causes. Time to response and MRD-negativity will be analyzed. Rate of response improvement during maintenance and conversion from MRD + to MRD –will be analyzed. OS will be analyzed using ITT population

Safety

Grade 3 or higher severe adverse events will be reported by GCP. All adverse events will be recorded to eCRF except adverse events < 2, which are already mentioned in the IB. Adverse events with grade ≥ 2 will be collected and analyzed. Treatment discontinuations and causes and possible dropouts and/or withdrawn patients with causes will be analyzed. Safety will be analyzed using ITT population

Efficacy analysis
The primary endpoint of this phase 2 study is the proportion of patients who obtain a MFC-MRD < 0.01% at any time during study protocol.

A'Hern single stage design.

P0=45%; p1=58%

2-sided significance level alpha=0.05; power=80%

Sample size=96, cut-off=52

Including a 20% drop-out rate the total sample is N=120

The proportion that is considered not interesting is p0=45%, while the desirable proportion is p1=58%.

The sample size is 120 patients and the cut-off for rejecting the null hypothesis is at least 52 patients achieving MRD-neg. So, if at least 52 patients out of 120 will be MRD-neg, then the null hypothesis is rejected and the true proportion is considered to be at 58%. But, if less than 52 successes are observed, the true proportion is less than 45% that is considered not interesting. Kaplan-Meier method will be used to estimate the survival distribution based on intention-to-treat population. All p-values will be reported as 2-sided.

Estimated accrual rate with planned 20 study sites is 18 months.

9.1.2 Stratification

All patients will receive the same induction and consolidation treatment. The autologous stem cell mobilization, harvesting and transplantation will be done by standard practice of sites. Patients will be stratified for maintenance based on risk stratification by FISH. Patients with del 17p with proportion of at least 60% by FISH, t(4;14), t(14;16), t(14;20) or +1q will receive ixazomib plus lenalidomide combination and standard-risk and low-risk patients will receive lenalidomide alone maintenance. This stratification is based on FISH findings at diagnosis. It is estimated that number of patients will be 60 for HR-group and 60 for SR-LR-group.

9.1.3 Populations for Analysis

Intention to treat (ITT) population includes all patients who have received at least a single dose of study drug. Efficacy evaluable population includes patients who received at least one dose of study drug and had at least one post-baseline response assessment. Safety and efficacy analyses will be performed based on ITT population.
9.1.4 Procedures for Handling Missing, Unused, and Spurious Data

Any spurious data will be abandoned. Missing data will be handled as missing data not with replacing it with a median parameter number.

9.1.5 Demographic and Baseline Characteristics

Baseline characteristics will include following parameters: age, sex, race, ECOG performance status, ISS stage (plasma albumin and serum-beta-2-microglobuline), paraprotein isotype, S- and U- paraprotein, S-FLC and ratio, hemoglobin, WBC, differential, platelet count, CRP, creatinine, calcium ionized, liver enzymes, LD, bone marrow morphology, bone marrow multiparameter flow cytometry, bone marrow cytogenetic analyses, FISH analyses t(4;14), t(14;16), t(11;14), t(6;14), t(8;14) (optional), +1q, 13/13q del, 1p loss; cytogenetic del17p.

Bone imaging will include skeletal ray or whole body low-dose CT, (MRI of spina and hip if clinically indicated).

9.1.6 Pharmacokinetics/Pharmacodynamics/Biomarkers

Pharmacokinetics or pharmacodynamics assessments are not included in this study. Bone marrow and blood samples will be collected at diagnosis and at relapse and skin biopsies at diagnosis will be collected from patients to biobank for later translational studies including in first line with specific funding sequencing, biomarker and drug sensitivity and resistance test analyses of high-risk patients.

9.1.7 Safety Analysis

For safety analyses all adverse events will be documented if observed, mentioned during open questioning or when spontaneously reported. All parameters included in the schedule of study investigations should be evaluated for possible adverse events reporting.

9.1.8 Interim Analysis

There is not any official interim analyses in this study. The IRd regimen is indicated to be efficient in RRMM and also in phase 1/2 studies for NDMM, so any early interim analyses will not be needed to lead an early stop of study. Preliminary analyses will be done to evaluate the responses after induction and 3 months after ASCT to compare them with the respective of FMG-MM02 study. There will be not any conclusions based on this data.
The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki, 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. National Ethics Committee and Finnish Medicines Agency will approve the study protocol and any substantial amendment. In accordance with the Declaration of Helsinki patients have the right to withdraw from the protocol at any time for any reason. The investigator also has the right to withdraw patients from the protocol in the event of intercurrent illness, adverse events and treatment failure after a prescribed procedure, protocol violations, cure, administrative reasons or other reasons. If a patient decides to withdraw from the protocol, all efforts will be made to complete and report the observations as thoroughly as possible. A complete final evaluation at the time of the patient’s withdrawal should be made together with the reason.

The clinical trial Sponsor-Principal Investigator is the physical person or legal entity which is interested in the performance of the trial, signs requests for authorization addressed to the National Ethics Committee (EC) and regulatory authority of Finland and is responsible for the trial, including its performance, initiation and completion. The Sponsor-Principal Investigator will be responsible for ensuring compliance with applicable legal guidelines. Investigators must agree with this protocol and know in detail the properties of the drug used in this clinical trial. Investigators must provide the patient with a patient information sheet and help him/her to understand the explanation provided. It is important to tell the patient that his/her participation in the study is completely voluntary and that it will not affect patient-physician relationship. In addition, it will be guaranteed that all people involved in the study will observe the confidentiality of any information related to the patient. All participants in the study are covered by the National Pharmaceutical Insurance Pool.

10.1 Patient information and consent

Written informed consent of patient is required before any study related procedure. The investigator should provide enough time for patient to discuss about all details of the study. All questions concerning the study will be answered to the satisfaction of the patient before possible obtaining of consent. The content of the patient information letter, informed consent form and any other written information provided to patients will be in compliance with ICH-GCP and other applicable regulations and should be approved by the Ethics Committee before use. Whenever new important information, relevant to the patients’ consent, will be available, the
patient information letter, informed consent and any other written information will be revised. Any revised informed consent form and written information should be approved by the Ethics Committee before use. The patient should be informed in a timely manner if new information becomes available that might be relevant to the patients’ willingness to continue participation in the study.

### 10.2 Patient confidentiality

Each patient is assigned a unique patient study number at registration. In study documents the patient’s identity is coded by patient study number. In some cases date of birth is also listed. The local investigator will keep a subject enrolment and identification log that contains the key to the code, the personal identification data linked to each patient study number. This data is filed at the investigational site and should only be accessed by the investigator and the supporting site staff or by representatives of the sponsor-investigator or a regulatory agency for the purpose of monitoring visits or audits and inspections. The Information and Consent Form also explains that for data verification purposes an authorized regulatory authority, or an ethics review board may require direct access to parts of the hospital or practice records relevant to the study including patients’ medical history.

### 10.3 Study insurance

Before the start of the study the Sponsor, Sponsor-Principal Investigator and Principal Investigator of each site and country (Estonia, Finland, Lithuania, Norway, Sweden) will ensure that adequate insurance for patients is in place covering losses due to death or injury resulting from the study, in accordance with applicable laws and regulations in each country where the trial is conducted. Adequate insurance for investigators and study staff will be ensured. The pharmaceutical company supplying drugs used in the study must have their own liability insurance.

### 10.4 Study monitoring

Independent staff from another institution or CRO company, not involved in the study, will perform monitoring of the study. Inclusion criteria, endpoints and all key test results according to the assessment schedule will be monitored to assure data quality. The investigator is required to permit direct access to the facilities where the study took place, source documents, CRFs and applicable supporting records of subject participation for audits and inspections by Ethics Committees and National Medicines Agency. The investigator should make every effort to be available for the audits and/or inspections.
10.5 Independent research board

In addition to Ethics Committee and regulatory authorities an Independent Research Board consisting of the members of Finnish Hematology Association, not participating in the study, will review every 6 months the course of the study.

10.6 Data and documents handling

Documents which are essential for evaluation of conduct of the study and the quality of data will be filed in such a manner that they are protected from accidental loss. The sponsor-investigator will file all national essential regulatory documents relevant to the overall conduct of the trial. Local investigators will file all essential documents relevant to the conduct of the trial on site. Essential documents will be retained for 15 years after the end of the trial and the final presentation of the study. Source documents of patients should be retained for 15 years after the end of the trial. After this time these documents will be handled by the site’s guidelines regarding medical records.

Patient’s medical file (e.g., medical records, ECG report, thorax x-ray, clinical laboratory examinations reports, and all other patient’s examinations results), quality of life questionnaires will be considered as source document.

An electronic Case Report Form (eCRF) will be used for data collection. The data is entered and corrected by investigator or authorized local study personnel and signed by the investigator. An electronic company has the ongoing service for backup. At the end of the study, the investigator will be provided with a copy of each participant’s data on a USB-stick support.

10.7 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. Biological samples shall be labelled with the patient’s identifying information on site. Samples that are shipped to another site e.g. central biobank for a later scientific research described in this protocol should be labelled only with a study code (study name or number and patients study code number).

10.8 Amendments

Any amendments to this protocol that seems appropriate, as the study proceeds (regarding safety, efficacy, conduction or scientific value of the study) will be agreed upon the
coordinating and/or principal investigator and investigator-sponsor. Amendments will be reviewed and approved by Celgene and Takeda before submission to the Ethical Committee. Amendments will be submitted to the Ethics Committee and the regulatory authority for written approval before the implementation of the amended version.

10.9 Annual reporting and updating

The Sponsor-Principal Investigator will submit a summary of the progress of the study to the accredited Ethics Committee and Competent Authority once a year. Information will be provided on the date of inclusion of the first patient, numbers of patients included and numbers of patients that have completed the study, serious adverse events/serious adverse reactions, treatment efficacy, possible problems, and amendments.

All potential serious breaches will be reported to the Competent Authority immediately. A serious breach is a breach of the conditions and principles of GCP in connection with the study or the protocol, which is likely to affect, to a significant degree, the safety or physical or mental integrity of the subjects of the study or the scientific value of the study.

10.10 Stopping rules/discontinuation criteria

The Sponsor-Principal Investigator may discontinue the study for medical reasons prior to inclusion of the intended number of patients. At the discretion of the Sponsor-Principal Investigator in collaboration with the Hospital District of Helsinki and Uusimaa and Nordic Myeloma Study Group Executive Board, the study may be discontinued for other reasons, prior to inclusion of the intended number of patients. A premature discontinuation of the study can be decided by the Sponsor-Investigator in the following cases:

- The study is not conducted in accordance with the procedures defined in the approved protocol (i.e. protocol deviations, failure to ensure the quality of the data collected or low rate of recruitment).

- If new information becomes available which results in changes in the risk/benefit assessment.

Conditions that may warrant termination of the study include but are not limited to the following:

- The discovery of an significant and unexpected or unacceptable risk to the patients enrolled in
- Failure of the Investigators to include patients in the study at an acceptable rate.

The Sponsor-Investigator will promptly notify all concerned Investigators, the Ethics Committee(s) and the regulatory authorities of the decision to terminate the study. The Sponsor-Investigator will provide information regarding the timelines of study termination and instructions regarding treatment and data collection of enrolled patients.

11. ADVERSE EVENTS

11.1 Definitions

11.1.1 Adverse Event Definition

Adverse event (AE) means any untoward medical occurrence in a patient or subject administered a pharmaceutical product; the untoward medical occurrence does not necessarily have a causal relationship with this 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 it is related to the medicinal product. This includes any newly occurring event, or a previous condition that has increased in severity or frequency since the administration of study drug.

All laboratory results defined in the study protocol are recorded to eCRF. All laboratory values out of reference range will be assessed by the investigator as an AE if that value leads to discontinuation or delay in treatment, dose modification, therapeutic intervention, or is considered to be a clinically significant by the investigator.

11.1.2 Serious Adverse Event Definition

Serious AE (SAE) means any untoward medical occurrence that at any dose:

- Results in death.
- Is life-threatening (refers to an AE in which the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe).
• Requires inpatient **hospitalization or prolongation of an existing hospitalization** (see clarification in the paragraph below on planned hospitalizations).

• Results in **persistent or significant disability or incapacity**. (Disability is defined as a substantial disruption of a person’s ability to conduct normal life functions).

• Is a **congenital anomaly/birth defect**.

• Is a **medically important event**. This refers to an AE that may not result in death, be immediately life threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, may jeopardize the patient, require medical or surgical intervention to prevent 1 of the outcomes listed above, or involves suspected transmission via a medicinal product of an infectious agent. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse; any organism, virus, or infectious particle (eg, prion protein transmitting Transmissible Spongiform Encephalopathy), pathogenic or nonpathogenic, is considered an infectious agent.

• Second primary malignancies will be monitored as events of interest and must be reported as serious adverse events regardless of the treatment arm the subject is in. This includes any second primary malignancy, regardless of causal relationship to IP (study drug[s]) or control, occurring at any time for the duration of the study, from the time of signing the informed consent up to the end of 10 years follow-up of study patients. Events of second primary malignancy must be considered as “Important Medical Event” even if no other serious criteria apply; these events must also be documented in the appropriate page(s) of the CRF and subject’s source documents. Documentation on the diagnosis of the second primary malignancy must be provided at the time of reporting as a serious adverse event (e.g., any confirmatory histology or cytology results, X-rays, CT scans, etc.)

Clarification should be made between a serious AE (SAE) and an AE that is considered severe in intensity (Grade 3 or 4), because the terms serious and severe are NOT synonymous. The general term **severe** is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor medical significance (such as a Grade 3 headache). This is NOT the same as **serious**, which is based on patient/event outcome or action
criteria described above, and is usually associated with events that pose a threat to a patient’s life or ability to function. A severe AE (Grade 3 or 4) does not necessarily need to be considered serious. For example, a white blood cell count of 1000/mm$^3$ to less than 2000 is considered Grade 3 (severe) but may not be considered serious. Seriousness (not intensity) serves as a guide for defining regulatory reporting obligations.

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 authorized medicinal product.

11.2 Procedures for Reporting Serious Adverse Events

AEs may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures. Any clinically relevant deterioration in laboratory assessments or other clinical finding is considered an AE. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event. For serious AEs, the investigator must determine both the intensity of the event and the relationship of the event to study drug administration.

AEs which are serious must be reported to Millennium Pharmacovigilance (or designee) from the first dose of study drug through 30 days after administration of the last dose of ixazomib. Any SAE that occurs at any time after completion of ixazomib treatment or after the designated follow-up period that the sponsor-investigator and/or sub-investigator considers to be related to any study drug must be reported to Millennium Pharmacovigilance (or designee). In addition, new primary malignancies that occur during the follow-up periods must be reported, regardless of causality to study regimen, for a minimum of three years after the last dose of the investigational product, starting from the first dose of study drug. All new cases of primary malignancy must be reported to Millennium Pharmacovigilance (or designee).

Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the trial are not to be considered AEs unless the condition deteriorated in an unexpected manner during the trial (e.g., surgery was performed earlier or
later than planned). All SAEs should be monitored until they are resolved or recovered with gradus 1 or are clearly determined to be due to a patient’s stable or chronic condition or intercurrent illness(es).

Since this is an investigator-initiated study, the principal investigator Raija Silvennoinen, also referred to as the sponsor-investigator, is responsible for reporting serious adverse events (SAEs) to any regulatory agency and to the sponsor-investigator’s EC or IRB.

Regardless of expectedness or causality, all SAEs (including serious pretreatment events) must also be reported in English to Millennium Pharmacovigilance (or designee):

**Fatal and Life Threatening SAEs and second primary malignancies** within 24 hours of the sponsor-investigator’s observation or awareness of the event

**All other serious (non-fatal/non life threatening) events** within 24 hours of the sponsor-investigator’s observation or awareness of the event

See below for contact information for the reporting of SAEs to Millennium Pharmacovigilance. The sponsor-investigator must fax or email the SAE Form per the timelines above. A sample of an SAE Form will be provided.
The SAE report must include at minimum:

- **Event term(s)**
- **Serious criteria**
- **Intensity of the event(s):** Sponsor-investigator’s or sub-investigator’s determination. Intensity for each SAE, including any lab abnormalities, will be determined by using the NCI CTCAE version 4.0 (v4.03. June 14, 2010), as a guideline, whenever possible. The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.

- **Causality of the event(s):** Sponsor-investigator’s or sub-investigator’s determination of the relationship of the event(s) to study drug administration.

Follow-up information on the SAE may be requested by Millennium.

Intensity for each SAE, including any lab abnormalities, will be determined by using the NCI CTCAE version 4.0 (see above), as a guideline, whenever possible. The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.

In the event that this is a multisite study, the sponsor-investigator is responsible to ensure that the SAE reports are sent to Millennium Pharmacovigilance (or designee) from all sites participating in the study. Sub-investigators must report all SAEs to the sponsor-investigator so that the sponsor-investigator can meet his/her foregoing reporting obligations to the required regulatory agencies and to Millennium Pharmacovigilance, unless otherwise agreed between the sponsor-investigator and sub-investigator(s).

Relationship to all study drugs for each SAE will be determined by the investigator or sub-investigator by responding yes or no to the question: Is there a reasonable possibility that the AE is associated with the study drug(s)?

Sponsor-investigator must also provide Millennium Pharmacovigilance with a copy of all communications with applicable regulatory authorities related to the study product(s), as soon as possible but no later than 4 calendar days of such communication.

<table>
<thead>
<tr>
<th>SAE reporting for</th>
<th>Sponsor-Principal Investigator</th>
<th>Raija Silvennoinen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helsinki University Hospital Finland</td>
<td>FAX number +358 9 471 71 897</td>
<td></td>
</tr>
</tbody>
</table>
SAE and Pregnancy Reporting Contact Information

US & Canada
Fax Number: 1-800-963-6290
Email: TakedaOncoCases@cognizant.com

Rest of World
Fax #: 1 202 315 3560

E-mail: TakedaOncoCases@cognizant.com

Suggested Reporting Form:

- SAE Reports will be sent simultaneously to Celgene and Takeda (template provided by Millennium)
- US FDA MedWatch 3500A:
- Any other form deemed appropriate by the sponsor-investigator

11.3 Procedures for Reporting Drug Exposure During Pregnancy and Birth Events

Takeda:

If a woman becomes pregnant or suspects that she is pregnant while participating in this study or within 90 days after the last dose, she must inform the investigator immediately and permanently discontinue study drug. The sponsor-investigator must immediately fax a completed Pregnancy Form to the Millennium Department of Pharmacovigilance or designee (see Section 11.2). The pregnancy must be followed for the final pregnancy outcome.

If a female partner of a male patient becomes pregnant during the male patient’s participation in this study, the sponsor-investigator must also immediately fax a completed Pregnancy Form to the Millennium Department of Pharmacovigilance or designee (see Section 11.2). Every effort should be made to follow the pregnancy for the final pregnancy outcome.

Suggested Pregnancy Reporting Form:

- Pregnancy Report Form (provided by Millennium)

Celgene: All pregnancies or suspected pregnancies occurring in either a female subject of childbearing potential or partner of childbearing potential of a male subject are immediately reportable events. The exposure of any pregnant female (e.g., caregiver, pharmacist, study
coordinator or monitor) is also an immediately reportable event. See also Chapter 7.8 Pregnancy.

11.4 Procedures for SUSAR reporting

The sponsor-investigator is responsible for reporting SUSARs to the regulatory authorities, the ethics committees and to the EMA EudraVigilance database. The Sponsor-Principal Investigator is also responsible to inform other involved investigators in the study about the SUSARs.

- A SUSAR resulting in death or judged as life threatening must be reported to regulatory authorities and the ethics committees within 7 days after the sponsor has been notified about the event. A full report has to be sent to the authorities within 15 days.

- A SUSAR, which is not resulting in death or is life-threatening, has to be reported to regulatory authorities and the ethics committees within 15 days after the Sponsor-Principal Investigator has been notified about the event. A full report has to be sent to the authorities as soon as possible.

To achieve this within given timelines the investigators are responsible for reporting the SAEs to the sponsor-investigator within 24 hours after having been notified about the events.

12. ADMINISTRATIVE REQUIREMENTS

12.1 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 should immediately contact Millennium (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 Millennium Quality representative.

<table>
<thead>
<tr>
<th>For Product Complaints,</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Phone: 1-844-N1-POINT (1-844-617-6468)</td>
</tr>
<tr>
<td>- E-mail: <a href="mailto:GlobalOncologyMedinfo@takeda.com">GlobalOncologyMedinfo@takeda.com</a></td>
</tr>
<tr>
<td>- FAX: 1-800-881-6092</td>
</tr>
<tr>
<td>- Hours: Mon-Fri, 9 a.m. – 7 p.m. ET (US)</td>
</tr>
</tbody>
</table>

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 Millennium Pharmacovigilance.
13. REFERENCES

9. Kumar S et al. ASH 2012 (Abstract 332)
10. Kumar S et al. IMW 2013 (Abstract P-230)
11. Richardson PG et al. EHA 2013 (Abstract P236)
12. Kumar SK, Berdeja JG, Niesvizky R et al. Safety and tolerability of ixazomib, an oral proteasome inhibitor, in combination with lenalidomide and dexamethasone in patients with previously untreated multiple myeloma: an open-label phase 1/2 study. Lancet Oncol 2014. Published online November 14, 2014 http://dx.doi.org/10.1016/S1470-2045(14)71125-8
13. Richardson PG et al. ASH 2013 (Abstract 535)
15. San Miguel J et al. EHA 2012 (Abstract 0293)
16. Kumar SK et al. ASH 2013 (Abstract 1944)
17. Moreau P, Masszi T, Grzasko N et al. Ixazomib, an investigational oral proteasome inhibitor (PI), in combination with lenalidomide and dexamethasone (IRd), significantly extends progression-free survival (PFS) for patients with relapsed and/or refractory multiple myeloma (RRMM): The phase 3 Tourmaline-MM1 Study (NCT01564537)


37. (NCT01790737) A Prospective Phase II Study to Assess Immunophenotypic Remission
After 3-drug Induction Followed by Randomized Stem Cell Mobilization, Autologous Stem Cell Transplantation (ASCT) and Lenalidomide Maintenance in Newly Diagnosed Multiple Myeloma


43. Kumar S, et al. Weekly Dosing of the Investigational Oral Proteasome Inhibitor MLN9708 in Patients with Relapsed and/or Refractory Multiple Myeloma: Results From a Phase 1 Dose-Escalation Study In 53rd ASH Annual Meeting and Exposition; 2011 10-13 Dec; San Diego, CA; p. abstr 816.


45. Kumar, S. et al. Phase 1/2 Study of Weekly MLN9708, an Investigational Oral Proteasome Inhibitor, in Combination with Lenalidomide and Dexamethasone in Patients with Previously Untreated Multiple Myeloma (MM) in 54th ASH Annual Meeting and Exposition. 2012. Atlanta, Georgia.

46. Richardson, P.G., et al. MLN9708, an investigational proteasome inhibitor, in combination with lenalidomide and dexamethasone in previously untreated multiple myeloma patients (pts): Evaluation of weekly and twice-weekly dosing in 17th EHA Annual Congress. 2012. Amsterdam, the Netherlands.


54. NCT01208662 DFCI/IFM
55. NCT01208766 HOVON/NMSG
61. CTCAE, Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (v4.03.June 14, 2010)
14. APPENDICES

14.1 Eastern Cooperative Oncology Group (ECOG) Scale for Performance Status

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal activity. Fully active, able to carry on all predisease performance without restriction</td>
</tr>
<tr>
<td>1</td>
<td>Symptoms but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work)</td>
</tr>
<tr>
<td>2</td>
<td>In bed &lt; 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.</td>
</tr>
<tr>
<td>3</td>
<td>In bed &gt; 50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.</td>
</tr>
<tr>
<td>4</td>
<td>100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair</td>
</tr>
<tr>
<td>5</td>
<td>Dead</td>
</tr>
</tbody>
</table>


14.2 Cockcroft-Gault Equation

For males:

\[
\text{Creatinine Clearance} = \frac{(140-\text{age[years]} \times \text{weight[kg]})}{72 \times (\text{serum creatinine[mg/dL]})} \quad \text{OR} \quad \frac{(140-\text{age[years]} \times \text{weight[kg]})}{0.81 \times (\text{serum creatinine[µmol/L]})}
\]

For females:

\[
\text{Creatinine Clearance} = 0.85 \times \frac{(140-\text{age[years]} \times \text{weight[kg]})}{72 \times (\text{serum creatinine[mg/dL]})} \quad \text{OR} \quad 0.85 \times \frac{(140-\text{age[years]} \times \text{weight[kg]})}{0.81 \times (\text{serum creatinine[µmol/L]})}
\]

IMWG CRAB criteria for study inclusion

This study includes patients only with evidence of organ damage; CRAB criteria

International Myeloma Working Group Criteria for Diagnosis


Multiple myeloma

<table>
<thead>
<tr>
<th>Clonal bone marrow plasma cells ≥ 10% or biopsy-proven bone or extramedullary plasmacytoma and any one or more of the following myeloma defining events</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myeloma defining events</strong></td>
</tr>
<tr>
<td>Evidence of end organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically:</td>
</tr>
<tr>
<td>1. Hypercalcemia: serum calcium &gt; 0.25 mmol/l higher than the upper limit of normal or &gt; 2.75 mmol/l</td>
</tr>
<tr>
<td>2. Renal insufficiency: creatinine clearance &lt; 40 ml/min or serum creatinine &gt; 177 µmol/l</td>
</tr>
<tr>
<td>3. Anemia: hemoglobin value of &gt; 20 g/l below the lower limit of normal, or a hemoglobin value &lt; 100 g/l</td>
</tr>
<tr>
<td>4. Bone lesions: one or more osteolytic lesions on skeletal radiography, CT or PET-CT</td>
</tr>
</tbody>
</table>

Any or more of the following biomarkers of malignancy:

1. Clonal bone marrow plasma cell percentage ≥ 60%
2. Involved:uninvolved serum free light chain ratio ≥ 100
3. > 1 focal lesions on MRI studies

New international staging system (ISS) (Greipp et al. JCO 2005; 23: 3412-3420)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Criteria</th>
<th>Median survival months</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Serum β2-microglobulin &lt; 3.5 mg/l Serum albumin ≥ 35 g/l</td>
<td>62</td>
</tr>
<tr>
<td>II</td>
<td>Not stage I or III*</td>
<td>44</td>
</tr>
<tr>
<td>III</td>
<td>Serum β2-microglobulin ≥ 5.5 mg/l</td>
<td>29</td>
</tr>
</tbody>
</table>

* There are two categories for stage II: serum β2-microglobulin < 3.5 mg/l but serum albumin < 35 g/l; or serum β2-microglobulin 3.5 to 5.5 mg/l irrespective of the serum albumin level.
### 14.5


<table>
<thead>
<tr>
<th>Response</th>
<th>Response criteria$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>sCR</td>
<td>CR as defined below plus Normal FLC ratio (0.26-1.65) and Absence of clonal cells in bone marrow$^b$ by immunohistochemistry or immunophenotyping$^c$</td>
</tr>
<tr>
<td>CR</td>
<td>Negative IFE of serum and urine and Disappearance of any soft tissue plasmacytomas and $&lt; 5%$ plasma cells in bone marrow$^b$ In patients in whom the only measurable disease is by sFLC levels, CR is defined as a normal FLC ratio (0.26-1.65) in addition to the CR criteria listed above</td>
</tr>
<tr>
<td>VGPR</td>
<td>Serum and urine M-protein detectable by IFE but not on electrophoresis or 90% or greater reduction in serum M-protein plus urine M-protein level $&lt; 100 \text{ mg per 24h}$ In patients in whom the only measurable disease is by sFLC levels, VGPR is defined as a $&gt; 90%$ decrease in the difference between involved and uninvolved sFLC levels</td>
</tr>
<tr>
<td>PR</td>
<td>$\geq 50%$ reduction of serum M-protein and reduction in 24-h urinary M-protein by $\geq 90%$ or to $&lt; 200 \text{ mg per 24 h}$ In patients in whom the only measurable disease is by sFLC levels, PR is defined as a $\geq 50%$ decrease in the difference between involved and uninvolved sFLC levels If serum and urine M-protein are unmeasurable, and sFLCs are also unmeasurable, $\geq 50%$ reduction in bone marrow plasma cells is required in place of M-protein, provided baseline percentage was $\geq 30%$ In addition to the above listed criteria, if present at baseline, a $\geq 50%$ reduction in the size of soft tissue plasmacytomas is also required</td>
</tr>
<tr>
<td>SD$^d$</td>
<td>Not meeting criteria for CR, VGPR, PR or progressive disease</td>
</tr>
</tbody>
</table>

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

$^a$ All response categories require two consecutive assessments made at anytime before the institution of any new therapy; all categories also require no known evidence of progressive or new bone lesions if radiographic studies were performed.

$^b$ Confirmation with repeat bone marrow examination not needed.

$^c$ Presence/absence of clonal cells is based upon the $\kappa/\lambda$ ratio. An abnormal $\kappa/\lambda$ ratio by immunohistochemistry and/or immunofluorescence requires a minimum of 100 plasma cells for analysis. An abnormal ratio reflecting presence of an abnormal clone is $\kappa/\lambda$ of $> 4:1$ or $< 1:2$.

$^d$ Not recommended for use as an indicator of response; stability of disease is best described by providing the time to progression estimates.

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

<table>
<thead>
<tr>
<th>Relapse subcategory</th>
<th>Relapse criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Progressive criteria</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Progressive disease: requires one or more of the following: Increase of ≥ 25% from lowest response value in serum M-component (the absolute increase must be ≥ 0.5 g/dl)&lt;sup&gt;b&lt;/sup&gt; and/or Increase of ≥ 25% from lowest response value in urine M-component (the absolute increase must be ≥ 200 mg/24 h) and/or In patients in whom the only measurable disease is by sFLC levels, increase of ≥ 25% from lowest response value in the difference between involved and uninvolved sFLC levels (absolute increase must be &gt; 100 mg/L) If serum and urine M-protein are unmeasurable, and sFLC are also unmeasurable, increase of ≥ 25% from lowest response value in bone marrow plasma cell percentage (absolute % must be ≥ 10%) Definite development of new bone lesions or soft tissue plasmacytomas or definitive increase in the size of existing bone lesions or soft tissue plasmacytomas Development of hypercalcemia (corrected serum calcium &gt; 11.5 mg/dl or 2.65 mmol/l) that can be attributed solely to the plasma cell proliferative disorder</td>
</tr>
<tr>
<td><strong>Clinical relapse</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Clinical relapse requires one or more of: Direct indicators of increasing disease and/or end organ dysfunction (CRAB features)&lt;sup&gt;§&lt;/sup&gt;. It is not used in calculation of time to progression or progression-free survival but is listed here as something that can be reported optionally or for use in clinical practice 1. Development of new soft tissue plasmacytomas or bone lesion 2. Definite increase in the size of existing plasmacytomas or bone lesions. A definite increase is defined as a 50% (and at least 1 cm) increase as measured serially by the sum of the products of the cross-diameters of the measurable lesion. 3. Hypercalcaemia (&gt; 2.65 mmol/l) (11.5 mg/dl) 4. Decrease in hemoglobin of ≥ 1.25 mmol/l (2 g/dl) 5. Rise in serum creatinine by 177 µmol/l or more (2 mg/dl or more) 6. Hyperviscosity</td>
</tr>
</tbody>
</table>
Relapse from CR\textsuperscript{a}

(To be used only if the end point studied is DFS)\textsuperscript{d}

Any one or more of the following
Reappearance of serum or urine M-protein by immunofixation or electrophoresis
In patients in whom the only measurable disease is by sFLC levels, reappearance of abnormal sFLC levels (absolute increase must be \( \geq 100 \) mg/L)
Development of \( \geq 5\% \) plasma cells in the bone marrow\textsuperscript{c}
Appearance of any other sign of progression (i.e., new plasmacytoma, lytic bone lesion, or hypercalcemia see above)

Abbreviations: CR, complete response; DFS, disease-free survival.
\textsuperscript{a} All relapse categories require two consecutive assessments made at anytime before classification as relapse or disease progression and/or the institution of any new therapy.
\textsuperscript{b} For progressive disease, serum M-component increases of \( \geq 10 \) g/l are sufficient to define relapse if M-component is \( \geq 50 \) g/l.
\textsuperscript{c} Relapse from CR has the 5% cutoff versus 10% for other categories of relapse.
\textsuperscript{d} For purposes of calculating time to progression and progression-free survival, CR patients should also be evaluated using criteria listed above for progressive disease

14.7 NEW YORK HEART ASSOCIATION CLASSIFICATION OF CARDIAC DISEASE (NYHA)

<table>
<thead>
<tr>
<th>Class</th>
<th>Functional Capacity</th>
<th>Objective Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.</td>
<td>No objective evidence of cardiovascular disease.</td>
</tr>
<tr>
<td>II</td>
<td>Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.</td>
<td>Objective evidence of minimal cardiovascular disease.</td>
</tr>
<tr>
<td>III</td>
<td>Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.</td>
<td>Objective evidence of moderately severe cardiovascular disease.</td>
</tr>
<tr>
<td>IV</td>
<td>Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.</td>
<td>Objective evidence of severe cardiovascular disease.</td>
</tr>
</tbody>
</table>

14.8 Multiparameter flow cytometry method standard operating procedure

**MM flow-MRD**

Panel configuration and antibody sources

<table>
<thead>
<tr>
<th>Tube</th>
<th>BV421</th>
<th>BV510</th>
<th>FITC</th>
<th>PE</th>
<th>PerCP-Cy5.5</th>
<th>PECy7</th>
<th>APC</th>
<th>APCC750</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CD138</td>
<td>CD27</td>
<td>CD38</td>
<td>CD56</td>
<td>CD45</td>
<td>CD19</td>
<td>CD117</td>
<td>CD81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5µL</td>
<td>10µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>2µL</td>
<td>10µL</td>
<td>6µL</td>
<td>2µL</td>
<td>10µL</td>
<td>5µL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cylgk</td>
<td>Cylgk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Target** | **Clone** | **Conjugate** | **Manufacturer** | **Ref. cat.**
--- | --- | --- | --- | ---
CD138 | M115 | BV421 | BD Biosciences | 562935
CD27 | O323 | BV510 | BioLegend | 302835
CD38 | Multi-epitope | FITC | Cytognos | CYT-38F2
CD55 | C5.9 | PE | Cytognos | CYT-56PE
CD45 | HI30 | PerCP-Cy5.5 | BioLegend | 304028
CD19 | J3-119 | PECy7 | Beckman Coulter | IM3628
CD117 | 104D2 | APC | BD Biosciences | 333233
Anti-Kappa | Polyclonal | APC | Dako | C0222
CD81 | M38 | APCC750 | Cytognos | CYT-81AC750
Anti-Lambda | Polyclonal | APCC750 | Cytognos | CYT-LAC750 (goal)
INTENDED USE

BulkLysis™ is an hypotonic lysing solution which provides osmotic lysis of the erythrocytes before immunofluorescence staining of peripheral blood or bone marrow aspirate samples.

SUMMARY AND EXPLANATION

The advanced multiparameter tools for the immunophenotypic characterization of different cell subsets refers not only to the instruments, but also to the reagents and detection limit (sensitivity). In order to achieve great sensitivity, starting with higher cell numbers is recommended. This can be achieved by using a fixative-free erythrocyte lysing solution before staining and that does not interfere with immunofluorescence cell staining.

REAGENT PROVIDED

BulkLysis™ is provided as 100 ml 10x concentrated solution containing ammonium chloride, potassium hydrogen carbonate and EDTA. This volume is sufficient for 20 tests (5 ml BulkLysis™ per test, for lysing each 2ml of sample). Reagent is not considered sterile.

STORAGE CONDITIONS

Concentrated BulkLysis™ is stable until the expiration date shown on the label when it is stored at 4-8 °C. The pH of the reagent may increase during unsuitable storage, which may affect cells scattered light in the FSC/SSC dot plot. The pH of the reagent should be between 7 and 7.4. If the pH is out this range, it should be adjusted by adding diluted solutions of HCl or NaOH.

WARNINGS AND RECOMMENDATIONS

1. The reagent is stable until the expiration date shown on the label if it is properly stored. Do no use after the expiration date shown on the label. If the reagents are stored in conditions different from those recommended, such conditions must be validated by the user.

2. Alteration in the appearance of the reagent, such as the precipitation or discoloration indicates instability or deterioration. In such cases, the reagent should not be used.

3. It is recommended handling the product with appropriate protective gloves and clothing, and its use by personnel sufficiently qualified for the procedures described. Avoid contact of samples with skin and mucous membranes. After contact with skin, wash immediately with plenty of water.

4. Use of the reagent with incubation times or temperatures different from those recommended may cause erroneous results. Any such changes must be validated by the user.

5. Any serious incident relating to the product must be reported to Cytognos S.L. as well as the competent professional authority of the Member State in which the user is established.
PROTOCOL

1. Dilute the concentrated BulkLysis™ solution (1:10) using distilled water.
2. Transfer no more than 2 ml of the sample containing at least 10 x 10^6 nucleated cells (peripheral blood or bone marrow aspirate collected on anticoagulant) to a 50 ml tube.
3. Fill the tube up to reach 50 ml volume using the diluted BulkLysis™.
4. Shake and incubate for 15 minutes on a laboratory roller mixer.
5. Centrifuge for 10 minutes at 800 xg speed.
6. Gently discard the supernatant using a vacuum pump or Pasteur pipette.
7. Add 2 ml of PBS + 0.5% (m/v) BSA + 0.09% (m/v) Sodium Azide and resuspend the cellular pellet. Fill up to 50 ml with the same washing buffer.

Note: In case you have a significant cell concentration, it is important to re-suspend the cell pellet with 2 ml PBS + 0.5% (m/v) BSA + 0.09% (m/v) Sodium Azide by mixing slowly and gently. That is, add the PBS slowly, little by little, and mix gently between lots. If it is necessary, you can re-suspend by using a pipette. Then, add PBS + 0.5% (m/v) BSA + 0.09% (m/v) Sodium Azide up to 50 ml. This should help in avoiding clumping.

8. Centrifuge for 5 minutes at 800 xg speed.
9. Gently discard the supernatant using a vacuum pump or Pasteur pipette.
10. Add 2 ml of PBS + 0.5% (m/v) BSA + 0.09% (m/v) Sodium Azide and resuspend the cellular pellet. Pass the cellular suspension into a 5ml flow cytometry tube. Add 2 ml of washing buffer into the 50 ml tube, mix it well and transfer to the 5ml tube.

Note: In case you have a significant cell concentration, it is important to re-suspend the cell pellet with 2 ml PBS + 0.5% (m/v) BSA + 0.09% (m/v) Sodium Azide by mixing slowly and gently. That is, add the PBS slowly, little by little, and mix gently between lots. If it is necessary, you can re-suspend by using a pipette. This should help in avoiding clumping.

11. Centrifuge for 5 minutes at 540 xg.
12. Discard the supernatant and resuspend the cellular pellet adjusting the volume in order to obtain 1x10^6 cells/μl.
13. Transfer the cells into an appropriate tube for staining procedures.

For more informations about the protocol please enter the EuroFlow website.

FLOW CYTOMETRY ANALYSIS

Check that the cytometer is correctly aligned and standardized for light dispersion and that the right compensation has been set following the instructions for each cytometer.

The figure below shows representative flow cytometry data on normal peripheral blood treated with BulkLysis™ only (prior to staining), other commercial lysis with fixative agent (after staining) and the combination of the two (BulkLysis™ prior to staining and fixative agent lysis after staining). This reagent sometimes separates the leukocyte subpopulations into two discrete populations with the same SSC characteristics, but different FSC (it has to be taken into account whenever specific population acquisition gates are to be used). The forward/side scatter characteristics might change compared to the classic image due to the lack of the fixative agent. In order to obtain the same FSC/SSC image as for whole blood normal stainings it is advisable to use a fixative lysing solution, after the incubation with the monoclonal antibodies.
Figure: Scatter characteristics of peripheral blood lysed with BulkLysis™ working solution and/or lysis with fixative agent (FSC threshold at 10 000; Image obtained using Infinicyt® software, Cytognos, Spain).

Troubleshooting: In general, after the BulkLysis™ with ammonium chloride the cell pellet obtained should be free of red blood cells. In some cases, it was observed a red cell pellet following the first centrifugation of the protocol, due to: the medical treatment status and type and/or the age of the sample from its extraction until its processing, but also related to deteriorated lysis solution (pH, below 20-C).

WARRANTY

This product is warranted only to conform to the quantity and contents stated on the label. There are no warranties that extend beyond the description on the label of the product. Cytognos’s sole liability is limited to either replacement of the product or refund of the purchase price.

EXPLANATION OF SYMBOLS

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PRODUCED BY

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