CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208462Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Ninlaro (ixazomib)

Date: November 13, 2015
To: File for NDA 208462
From: John K. Leighton, PhD, DABT
Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting and labeling reviews for Ninlaro conducted by Dr. Place, and secondary memorandum and labeling provided by Dr. Sheth. I concur with Dr. Sheth's conclusion that Ninlaro may be approved for the proposed indication.

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/s/

JOHN K LEIGHTON
11/13/2015

MEMORANDUM

Date: November 09, 2015
From: Christopher Sheth, PhD
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
NDA: 208462
Drug: Ninlaro (ixazomib)
Indication: Treatment of patients with multiple myeloma who have received at least one prior therapy
Applicant: Millennium Pharmaceuticals, Inc.

Ixazomib is a small molecule reversible inhibitor of the chymotrypsin-like activity of the beta 5 subunit of the 20S proteasome, being developed as a treatment for patients with multiple myeloma who have received at least one prior therapy. Ixazomib will be formulated into capsules of Ninlaro of 2.3, 3, and 4 mg strengths. Ninlaro will be administered orally once a week on Days 1, 8, and 15 of a 28-day treatment cycle, in combination with lenalidomide and dexamethasone.

The pharmacology and toxicology studies reviewed included primary pharmacodynamics, genotoxicity, safety pharmacology, repeat dose toxicology (6-month rat and 9-month dog), and embryo-fetal developmental toxicity in rats and rabbits. With regards to the pharmacology of ixazomib, the drug induced apoptosis of multiple myeloma cell lines in vitro and demonstrated cytotoxicity against myeloma cells from patients who had relapsed after multiple prior therapies, including bortezomib, lenalidomide, and dexamethasone. The combination of ixazomib and lenalidomide demonstrated synergistic cytotoxic effects in multiple myeloma cell lines. Additionally, ixazomib demonstrated antitumor activity in vivo in a mouse multiple myeloma tumor xenograft model. The Established Pharmacological Class of "proteasome inhibitor" was determined to be both scientifically valid and clinically meaningful for ixazomib.

In multi-cycle general toxicity studies conducted in dogs, the principal target organs included the nervous system. Nervous system effects were primarily seen in dogs at oral doses greater than or equal to 0.1 mg/kg (2 mg/m^2) and included microscopic findings of minimal to mild neuronal degeneration of the sympathetic, dorsal root, peripheral autonomic (salivary gland), end organ ganglia, and minimal secondary axonal/nerve fiber degeneration of the peripheral nerves and ascending tracts in the dorsal columns of the spinal cord. In the 9-month study (10 cycles) in dogs where the dosing regimen mimics the clinical regimen (28-day cycle), microscopic neuronal effects were generally minimal in nature and only observed at 0.2 mg/kg (4 mg/m^2; $AUC_{0\text{-}168}$ = 1940 hr*ng/mL). The majority of target organ findings (e.g., in the gastrointestinal tract, lymphoid tissue, and nervous system) partially or completely recovered following discontinuation of treatment, except for the neuronal findings in the lumbar dorsal root ganglion and dorsal column.

The Applicant's proposal for Section 8 of the label is consistent with the Pregnancy and Lactation Labeling Rule. Ninlaro can cause fetal harm (b) (4)

(b) (4) Ixazomib caused embryo-fetal toxicity in pregnant rats and rabbits at doses resulting in exposures (b) (4) observed in patients receiving the recommended dose. (b) (4)

(b) (4)

The nonclinical studies needed to support product labeling were reviewed by Dr. Emily Place. The nonclinical findings are summarized in the "Executive Summary" of the NDA review and reflected in the product label.

Recommendation: I concur with the pharmacology/toxicology reviewer that from a nonclinical perspective, Ninlaro may be approved and that no additional nonclinical studies are needed to support approval of Ninlaro in patients with multiple myeloma who received at least one prior therapy.

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/s/

CHRISTOPHER M SHETH
11/09/2015

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	208462
Supporting document/s:	1
Applicant's letter date:	July 10, 2015
CDER stamp date:	July 10, 2015
Product:	Ninlaro (ixazomib)
Indication:	Patients with multiple myeloma who have received at least one prior therapy
Applicant:	Millenium Pharmaceuticals Inc.
Review Division:	Division of Hematology Oncology Toxicology (DHOT) for Division of Hematology Products (DHP)
Reviewer:	Emily Place PhD MPH
Supervisor/Team Leader:	Chris Sheth PhD
Division Director:	John Leighton PhD, DABT (DHOT) Ann Farrell MD (DHP)
Project Manager:	Jacquin Jones MS BSN

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 208462 are owned by Millenium Pharmaceuticals Inc. or are data for which Millenium Pharmaceuticals Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 208462 that Millenium Pharmaceuticals Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 208462.

Reference ID: 3844453

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1 Executive Summary

1.1 Introduction

Ixazomib is a second-generation proteasome inhibitor that reversibly binds and inhibits the chymotrypsin-like activity of the β5 subunit of the 20S proteasome. Nonclinical pharmacology and toxicology studies have been submitted and reviewed to support the approval of Ninlaro for the treatment of patients who have received at least one prior therapy. The drug will be administered at a recommended dose of 20 mg once a week on Days 1, 8, and 15 of a 28-day treatment cycle by oral administration.

1.2 Brief Discussion of Nonclinical Findings

Ixazomib is a proteasome inhibitor similar to the previously approved Velcade (bortezomib). Upon (b) (4) the citrate ester form of the drug, MLN9708, is hydrolyzed into the active boronic acid moiety, MLN2238. Ixazomib reversibly binds to the active threonine reside in the β5 portion of the 20S proteasome, inhibiting its chymotrypsin like activity. A series of non-GLP studies have been conducted with ixazomib to investigate its pharmacology. In vitro cell culture-based assays confirmed that ixazomib does target the β5 subunit at its cymotryptic site and prevents proteasome mediated degradation. Ixazomib also has activity against the β5i isoform, of the immunoproteasome expressed in hematological cells. In vitro, ixazomib induces caspase mediated apoptosis in multiple myeloma cell lines. In multiple myeloma cell lines, ixazomib also had a synergistic effect on cytotoxicity in combination with lenalidomide. Ixazomib also induces cytotoxicity in cells from patients with multiple myeloma refractory to lenalidomide and dexamethasone. In vivo pharmacology studies using a human myeloma xenograft SCID mouse model following treatment with ixazomib confirmed dose-dependent proteasome inhibition by modulation of β5 subunit activity, tumor cell protein levels of GADD34 and caspase-3 (protein upregulated as parts of the unfolded protein response pathway and apoptosis). Ixazomib treatment of SCID mice inoculated with human myeloma tumor xenografts also reduces tumor volumes.

Stand-alone safety pharmacology studies were not conducted for the respiratory or nervous system. In the repeat-dose toxicology studies, ixazomib exposure did not result in clinical signs of toxicity, there were no toxicologically significant effects on the respiratory systems of rats or dogs, and there were no acute nervous system-related effects (as assessed in part by a functional observational battery (FOB) incorporated into the 9 month dog study). There were chronic toxicities of the nervous system in dogs (see Section 6). The IC_{50} in the hERG assay for ixazomib was 59.6 μM suggesting weak inhibition of the potassium channel. No adverse effects on cardiovascular parameters were observed in dogs administered oral ixazomib.

Based on the data collected in general toxicology studies, there were no gender differences in exposure to oral ixazomib, and increases in C_{max} and AUC values were dose proportional. Intravenous administration of 2 mg/kg ixazomib and oral administration of 6 mg/kg ixazomib resulted in similar plasma area under the

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concentration-time curves from the time 0 to 24 hours (AUC_{0-24hr}). Tissue distribution was extensive following administration of oral ixazomib. The highest exposure (by C_{max} or AUC) was in the urinary bladder, lymph node, spleen, adrenal glands, kidney (renal cortex and medulla), bile, GI tract, and liver. The longest observed half-life was for the urinary bladder with a $t_{1/2}$ of up to 946 hours. Upon [redacted] (b) (4), ixazomib citrate ester (MLN9708) is metabolized to the active boronic acid form of the drug, (MLN2238). The elimination half-life after oral dosing in nonclinical species ranged from 30 to 72 hours.

The general toxicology studies were conducted in the rat and dog via oral (gavage). All studies relevant to an approvability determination were conducted in compliance with Good Laboratory Practices (GLP).

General Toxicology studies

Nonclinical findings in the rat and dog show that ixazomib targets the gastrointestinal tract, hematopoietic/lymphatic systems, and nervous system (dog only). Findings include:

- Hematopoietic/lymphatic systems: lymphoid necrosis and depletion in lymph nodes and spleen correlating with increased absolute counts for total white blood cells, neutrophils, monocytes, and lymphocytes (rats only).
- Gastrointestinal tract: epithelial hyperplasia, neutrophilic infiltrates (stomach, large and small intestines, peyers patch, mesenteric lymph nodes), single-cell necrosis in the lamina propria of the small and large intestines, and erosion in the stomach.
- Nervous system (dogs only): neuronal degeneration of the sympathetic, dorsal root, peripheral autonomic, and end organ ganglia, and nerve fiber degeneration of the peripheral nerves and ascending tracts in the dorsal columns of the spinal cord. Changes present at recovery in high dose animals included nerve fiber degeneration of the dorsal root ganglia and an increase in axonal degeneration in the dorsal columns of the spinal cord.

Safety concerns stem from the steep dose-toxicity relationship with ixazomib in the chronic rat and dog toxicology studies. There was a less than 1.5-fold separation in systemic exposure between the limit dose and a dose associated with mortality due to drug-induced injury of the liver and gastrointestinal tract in the rat. In dogs, there was an approximately 2-fold separation in exposure between the no observable adverse effect level (NOAEL) and doses associated with extensive neuronal pathology in the central nervous system (CNS) and peripheral nervous system (PNS).

Genotoxicity

Ixazomib was non-mutagenic in the in vitro bacterial reverse mutation assay (AMES test). Ixazomib was not clastogenic in a bone marrow micronucleus assay in mice. Ixazomib showed clastogenic activity (structural chromosomal aberrations) in the in vitro chromosomal aberration assay in human peripheral blood lymphocytes in the presence

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or absence of an exogenous metabolic activation system. Ixazomib was negative for DNA damage in a Comet assay in mouse lymphoma cells.

Reproductive and Development Toxicity
Ixazomib was teratogenic in the rat and rabbit. In pregnant rat (0.6 mg/kg; 3.6 mg/m^2) dose range-finding studies, there were decreases in fetal weights, decreased fetal viability, and increased post-implantation losses (at 2.5 times the human AUC when given 4 mg orally, in the rat respectively). In pregnant rabbit (1.0 mg/kg; 12 mg/m^2) dose range-finding studies, there were decreases in fetal weights, decreased fetal viability, and increased post-implantation losses (at 3.2 times the human AUC when given 4 mg orally, in the rabbit). In the definitive rabbit study, increases in fetal skeletal variations/abnormalities (caudal vertebrae, number of lumbar vertebrae, full supernumerary ribs, asymmetric sternebrae, shortened tail) were observed at doses greater than or equal to 0.3 mg/kg (3.6 mg/m^2), which were also associated with maternal toxicity. The dose of 0.3 mg/kg (3.6 mg/m^2) in rabbits is approximately 1.9 times the human AUC when given 4mg orally. Thus, administration of ixazomib during pregnancy may pose a risk to the human fetus. The Applicant proposed a conservative post treatment use of 90 days contraception, based on potential effects of the drug and taking into consideration its long half-life. The agency does not object to the proposal.

1.3 Recommendations

1.3.1 Approvability

Recommended for approval. The nonclinical studies submitted to this NDA provide sufficient information to support the use of Ninlaro for the treatment of patients with multiple myeloma who have received at least one prior therapy.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The content for the labeling of ixazomib is contained in this review. Based on the nonclinical data submitted in the NDA, the Established Pharmacological Class (EPC) of “proteasome inhibitor” was determined to be both clinically meaningful and scientifically valid for ixazomib.

2 Drug Information

2.1 Drug

CAS Registry Number	1201902-20-3
Generic Name	Ixazomib citrate
Code Names	MLN9708 (ixazomib citrate); MLN2238 (ixazomib)
Chemical Name	B-[(1*R*)-1-[[2-[(2,5-dichlorobenzoyl)amino]acetyl]amino]-3-methylbutyl] boronic acid
Molecular formula/ Molecular Weight	$C_{20}H_{23}BCl_2N_2O_9$/ 517.12 g/mol; $C_{14}H_{19}BCl_2N_2O_4$ /361.03 g/mol

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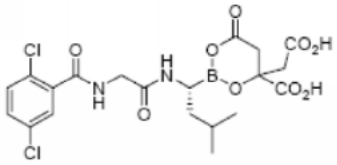
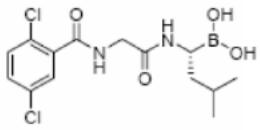
Structure or Biochemical Description	 MLN9708 (ester form)  MLN2238 (boronic acid)
Pharmacologic class	Proteasome inhibitor

Ixazomib (MLN2238) refers to the biologically active boronic acid form of the drug substance. The drug is administered as a stable citrate ester, ixazomib citrate (MLN9708). Under physiological conditions ixazomib citrate rapidly hydrolyzes to ixazomib.

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND (b) (4), IND104482, (b) (4)

2.3 Drug Formulation

The ixazomib capsule formulation in strengths of 2.3, 3.0, and 4.0 mg will be available, consisting of the drug substance (ixazomib citrate), microcrystalline cellulose, talc, and magnesium stearate.

Table 1. Ixazomib drug formulation (capsules)

Components	Function	Reference to Standards [a]	mg per capsule [w/w%] 2.3	3.0	4.0
Ixazomib citrate (equivalent to ixazomib)	API	In-house	(b) (4)		
Microcrystalline cellulose	(b) (4)	USP/NF, Ph.Eur., JP			
Talc		USP/NF, Ph.Eur., JP			
Magnesium stearate		USP/NF, Ph.Eur., JP			
Total weight					

(Excerpted from the submission)

2.4 Comments on Novel Excipients

All excipients are compendial.

2.5 Comments on Impurities/Degradants of Concern

Impurities	Proposed specification	Qualification analysis	Qualified (Y/N)
(b) (4)			

(b) (4)

Exposures for elemental impurities were calculated based on 4 mg maximum daily dose of ixazomib. Based on the 4 mg dose, each elemental impurity (b) (4) with the proposed acceptance criteria of ≤ (b) (4) ppm will have an exposure ≤ (b) (4) µg/day. Therefore, all of the impurities are acceptable.

2.6 Proposed Clinical Population and Dosing Regimen

Ninlaro will be administered in combination with lenalidomide and dexamethasone for the treatment of patients with multiple myeloma who have received at least one prior therapy. The drug is administered orally by capsule. The recommended starting dose of Ninlaro is 4 mg (one capsule), once a week on Days 1, 8, and 15 of a 28-day treatment cycle. The recommended starting dose of lenalidomide is 25 mg taken daily on Days 1 through 21 of a 28-day treatment cycle. The recommended starting dose of dexamethasone is 40 mg taken on Days 1, 8, 15, and 22 of a 28-day treatment cycle.

Table 2. Dosing schedule for Ninlaro with lenalidomide and dexamethasone

Dosing Schedule: NINLARO taken with Lenalidomide and Dexamethasone

✔ Take medicine

28-Day Cycle (a 4-week cycle)								
	Week 1		Week 2		Week 3		Week 4	
	Day 1	Days 2-7	Day 8	Days 9-14	Day 15	Days 16-21	Day 22	Days 23-28
NINLARO	✔		✔		✔			
Lenalidomide	✔	✔ Daily	✔	✔ Daily	✔	✔ Daily		
Dexamethasone	✔		✔		✔		✔	

(Excerpted from the submission)

2.7 Regulatory Background

NDA 208462 was submitted on July 10, 2015 for the treatment of patients with multiple myeloma who have received at least one prior therapy.

3 Studies Submitted

3.1 Studies Reviewed

Study Title	Study No.	Module
Primary Pharmacodynamics		
In vitro Pharmacology		
Enzymology and Cell Biology of MLN2238	RPT-01200 Amendment 2	4.2.1.1
Proteasome beta5- and beta2-Site Inhibition by Ixazomib and Bortezomib in HCT116 Cells Measured by Proteasome-Glo Assay	MLN9708-28351	4.2.1.1
Effects on in vitro viability of human myeloma cell lines	MLN9708-27528	4.2.1.1
Effects of ixazomib in combination with lenalidomide on in vitro viability of human myeloma cell lines	MLN9708-30663	4.2.1.1
In vivo Pharmacology		
A Pharmacokinetic/Pharmacodynamic Study of Ixazomib After A Single Oral or Intravenous Administration to MM.1S Multiple Myeloma Xenograft-Bearing Female CB-17 SCID Mice	MLN9708-24699	4.2.1.1
Antitumor Activity of Ixazomib (MLN2238) Administered Orally Twice Weekly in MM.1S Multiple Myeloma Xenograft-Bearing Female CB-17 SCID Mice	MLN9708-24176	4.2.1.1
Safety Pharmacology		
In Vitro Binding Study of ML00604174 (MLN2238) to the hERG Channel in HEK-293 Cells	RPT-01141	4.2.1.3
Safety pharmacology studies of MLN9708: effects on the cardiovascular system in conscious dogs	MLN9708-24953	4.2.1.3
Pharmacokinetics		
Absorption		
Pharmacokinetics of MLN2238 after oral administration of MLN9708 capsules to non-naive male beagle dogs	RPT-01218	4.2.2.2
Distribution		
Tissue Distribution via Quantitative Whole-Body Autoradiography in Male Long- Evans Rats Following a Single Oral Gavage Administration of [^{14}C] MLN9708 and Human Radiation Dosimetry Estimation	96N-1206	4.2.2.2
Metabolism		
Pharmacokinetics, Metabolism, Mass Balance, And Excretion Routes Of [14C]MLN9708 In Male Sprague-Dawley Rats Following A Single PO Dose	MILR1800R2A	4.2.2.2
Repeat dose toxicology: *(including supportive toxicokinetics)*		
A 6-Month Oral Gavage Toxicity Study of MLN9708 with a 2-Week Recovery Period in Sprague Dawley Rats	(b) (4)-416165	4.2.3.2
A 9-month oral gavage toxicity study of MLN9708 with a 2- week recovery period in Beagle dogs	(b) (4)-416164	4.2.3.2
Genotoxicity		

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Study Title	Study No.	Module
Salmonella E.coli/Mammalian Microsome Reverse Mutation Assay	MBR08-152	4.2.3.3
In vitro Chromosome Aberration Test in Cultured Human Peripheral Blood Lymphocytes	MBR12-367	4.2.3.3
MLN9708: A Single-Dose Oral (Gavage) Bone Marrow Micronucleus Evaluation in CD-1 Mice	WIL-416152	4.2.3.3
Comet assay of MLN-9708 for injection in mice	B130357	4.2.3.3
Reproductive and development Toxicity		
Range finding study for effects of MLN9708 on Embryo-fetal development in rats	13-07/te	4.2.2.4
Effects of MLN 9708 on Embryo-fetal development in Rats	13-155/te	4.2.2.4
An Oral (Gavage) Dose Range-Finding Study of MLN9708 on Embryo/Fetal Development in Rabbits	(b) (4)-416156	4.2.2.4
Effects of MLN 9708 on Embryo-fetal development in Rabbits	14-027/te	4.2.2.4

3.2 Studies Not Reviewed

Study Title	Study No.	Module
Pharmacology		
In vitro Pharmacology		
Customized Profile Screening Profile for Millennium Pharmaceuticals	T0-07-4203	4.2.1.1
In Vitro Inhibition of Proteasome Activity by ML00701207, an MLN9708 Enantiomer	MLN9708-31263	4.2.1.1
In vivo pharmacology		
Antitumor Activity of Ixazomib (MLN2238) Administered Intravenously Twice Weekly and Once Weekly in MM.1S Multiple Myeloma Xenograft-Bearing Female CB-17 SCID Mice	MLN9708-24177	4.2.1.1
Effect of MLN9708 on antibody production in a rat model of T cell-dependent antigen response	MLN9708-24528	4.2.1.1
A Pharmacokinetic/Pharmacodynamic Study of Ixazomib After A Single Oral or Intravenous Administration to MM.1S Multiple Myeloma Xenograft-Bearing Female CB-17 SCID Mice	MLN9708-24699	4.2.1.1
Effect of MLN9708 on autoantibody production in MRL/lpr lupus mice	MLN9708-24739	4.2.1.1
In Vivo Antitumor Activity and Pharmacodynamic Response of Bortezomib and MLN2238 in WSU-DLCL2 Subcutaneous Diffuse Large B Cell Lymphoma Xenografts in Female SCID Mice	RPT-01121	4.2.1.1
A Pharmacokinetic/Pharmacodynamic Study of MLN2238 (18 mg/kg) After an Intravenous Administration to CWR22 Tumor-Bearing Male SCID Mice	RPT-01161	4.2.1.1
A Pharmacokinetic/Pharmacodynamic Study of MLN2238 (14 mg/kg) After an Intravenous Administration to Male CWR22 Tumor-Bearing SCID Mice	RPT-01162	4.2.1.1
A Pharmacokinetic/Pharmacodynamic Study of MLN2238 (2 and 4 mg/kg) After a Subcutaneous Administration to CWR22 Tumor-Bearing Male SCID Mice	RPT-01163	4.2.1.1
In Vivo Antitumor Activity and Pharmacodynamic Response of MLN2238 and Bortezomib in WSU-DLCL2 Subcutaneous Diffuse	RPT-01165	4.2.1.1

Reference ID: 3844453

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(b) (4)

3.3 Previous Reviews Referenced

IND (b) (4), IND104482, (b) (4)

4 Pharmacology

4.1 Primary Pharmacology

MLN9708 is a second-generation proteasome inhibitor similar to the previously approved Velcade (bortezomib). Upon (b) (4) the citrate ester form of the drug, MLN9708, is hydrolyzed into the active boronic acid moiety, MLN2238. Ixazomib reversibly binds to the (b) (4) in the β5 portion of the 20S proteasome, inhibiting its chymotrypsin like activity. A series of non-GLP studies have been performed with ixazomib to investigate its pharmacology. In vitro cell culture-based assays confirmed that ixazomib does target the β5 subunit at its cymotryptic site and prevents proteasome mediated degradation. Ixazomib also has activity against the β5i isoform, of the immunoproteasome expressed in hematological cells. In vitro ixazomib induces caspase mediated apoptosis in multiple myeloma cell lines. Ixazomib also induces cytotoxicity in cells from patients with multiple myeloma refractory to lenalidomide and dexamethasone. Ixazomib was cytotoxic in culture to primary cultures of cells harvested from multiple myeloma patients who had relapsed after prior therapies. In multiple myeloma cell lines, ixazomib also had a synergistic effect on cytotoxicity in combination with lenalidomide as determined by both combination index and non-linear blending. In vivo assays looking at β5 subunit activity, tumor cell protein levels of GADD34 and caspase-3 (protein upregulated as parts of the unfolded protein response pathway and apoptosis) further confirm that ixazomib treatment results in dose-dependent proteasome inhibition. Furthermore, the studies indicate that ixazomib treatment of SCID mice inoculated with human myeloma tumor xenografts reduces tumor volumes.

Ixazomib enzymology: ixazomib inhibits the chymotrypsin-like activity of the β5 subunit of the 20S proteasome

Study no: MLN9708-28351, RPT-01200 Amendment 2

Methods The activity of ixazomib against the 20S proteasome subunits, β1 (caspase-like), β2 (trypsin-like), and β5 (chymotrypsin-like) and β5i (isoform for the immunoproteasome) proteolytic sites was determined using fluorogenic substrates in biochemical microtiter plate-based assays. IC_{50} values and inhibition dissociation constant were determined for ixazomib. The activity of ixazomib was also screened against 7 serine proteases and 2 cysteine proteases, as this class of peptidyl boronic acids are known to serine proteases with sequence-dependent activity.

Results

Ixazomib inhibited 20S proteasome activity in vitro; it had preferential activity by binding the β5 site of the 20S proteasome (IC_{50} of 3.4 nM: [CI] = 2.8 - 4.1 nM; n = 3). Ixazomib was approximately 10- and 1000-fold less potent against the β1 and β2 sites, respectively (IC_{50} = 31 and 3500 nM, respectively; n = 1 for both). Ixazomib also showed inhibition of the β5i subunit of the 20S immunoproteasome (an isoform of the 20S proteasome found in hematopoietic cells). The dissociation constant (Ki) was 0.4 nM for the β5i site which is similar to the Ki for the β5 site, 0.93 nM. The concentrations of activity and differences in selectivity of ixazomib for the proteasome active sites is similar to that of bortezomib, however bortezomib does have more preferential activity towards the β1 and β2 subunits of the 20S proteasome. In measuring selectivity against screened serine proteases, elastase was inhibited by ixazomib (IC_{50} = 19 μM), at a concentration more than 5000-fold that of the IC_{50} for the 20S proteasome β5 site (3.4 nM). The overall selectivity for elastase by ixazomib is related to elastases preference for a leucine at the P1 position.

Table 3. Ixazomib protease selectivity

Protease	IC_{50} (μM)
Chymotrypsin	> 100 (n = 2)
Trypsin	> 100
Elastase	19 (7.8 – 47, n = 3)
Thrombin	> 100
Plasmin	> 100
tPA	> 100
CFbetaXIIa	> 100
Cathepsin B	> 100 (n = 2)
Cathepsin L	> 100

Source: Report RPT-01200 Amendment 2.

CFbetaXIIa = F12 coagulation factor XII (Hageman factor); IC_{50} = concentration producing 50% inhibition; tPA = tissue plasminogen activator.

Note: Results are reported as geometric mean (95% confidence interval [CI], number of experiments). Data without CI are single determinations.

(Excerpted from the submission)

Reference ID: 3844453

Table 4. Summary of enzymology comparison of ixazomib and bortezomib

Biochemical Assays	Ixazomib	Bortezomib
K_i (nM)		
β5	0.93 (0.64 – 1.4, n = 3)	0.55 (0.34 – 0.89, n = 3)
β5i	0.4	0.2
IC_{50} (nM)		
β5	3.4 (2.8 – 4.1, n = 3)	2.4 (2.0 – 2.9, n = 45)
β2	3500	1200
β1	31	24 (14.5 – 40, n = 12)

Source: Report RPT-01200 Amendment 2.

IC_{50} = concentration producing 50% inhibition; K_i = inhibition dissociation constant.

Note: Results are reported as geometric mean (95% confidence interval [CI], number of experiments). Data without CI are single determinations.

(Excerpted from the submission)

Ixazomib inhibits the proteasome in cultured human tumor cells
Study no: MLN9708-28351, RPT-01200 Amendment 2

Methods
Three separate cell based assays were conducted to determine the ability of ixazomib to inhibit the proteasome and prevent proteasome dependent degradation. This was carried out using [redacted] (b) (4) cell-based assay to measure the kinetics of inhibition and recovery of chymotrypsin-like (β5 site) activity in live cells; by examining the effects of ixazomib on a direct proteasome substrate (NF-KB Luciferase assay); and by using the 4 x ubiquitin-luciferase reporter to directly monitor the degradation of polyubiquitinated luciferase by the proteasome.

Results
Using the Proteasome-Glo cell based assay, the IC_{50} for ixazomib and bortezomib after 1 hour of treatment in Calu-6 cells was 9.7 and 3.0 nM respectively which was approximately comparable. Recovery of proteasome activity after the brief exposure to and following washout (4 hours) of ixazomib and bortezomib (1 μM) was performed in the same assay system. Prior to washout, MLN2238 and bortezomib inhibited proteasome activity 92.9% and 96.55% respectively compared to untreated controls, but while bortezomib treated cells recovered only 20% of their activity, MLN2238 recovery was 69%. Ixazomib was active when used in an assay with the 4 x Ub-Luc reporter with producing a stimulatory maximum effect (E_{max}) of 265-fold in the MDA-MB-231-4 x Ub-Luc cell line, yielding a half-maximal response (EC_{50}) of 525 nM. In comparison, bortezomib yielded an E_{max} of 370-fold and an EC_{50} of 310 nM. When examining the effect of ixazomib on a proteasome substrate, using NF- KB activation by TNF-α, ixazomib almost completely inhibited (99.3%) activation in the NF- KB - Luc assay in HEK-293 cells, with an inhibitory concentration (IC_{50}) of 55 nM, compared to IC_{50} of 33 nM for bortezomib.

Reference ID: 3844453

Table 5. Ixazomib proteasome inhibition in cultured cells

Cell-Based Assay	Ixazomib	Bortezomib
Calu-6 Proteasome-Glo™[a] IC_{50} (nM)[b]	9.7	3.0
Calu-6 Proteasome-Glo (% Activity)[b, c]		
t = 4 hours, no washout	7.1 (3.6 – 10.6, n = 5)[d]	3.5 (2.0 – 4.9, n = 5)[c]
t = 4 hours, washout	69 (66 – 71, n = 5)[c]	20 (18 – 23, n = 5)[c]
MDA-MB-231 4 × Ub-Luc EC_{50} (nM)	525 (330 – 840, n = 4)	310 (230 – 400, n = 29)
E_{max} (fold stimulation)	265 (160 – 370, n = 4)[c]	370 (330 – 410, n = 29)[c]
HEK-293 NF-κB–Luc IC_{50} (nM)	55 (33 – 91, n = 7)	33 (27 – 40, n = 23)
Maximum inhibition (%)	99.3 (99.0 – 99.6, n = 7)[c]	99.6 (99.3 – 100, n = 23)[c]

Source: Report RPT-01200 Amendment 2.

EC_{50} = concentration producing half-maximal response; E_{max} = maximum effect; IC_{50} = concentration producing 50% inhibition

Note: Results are presented as the geometric mean (95% confidence interval [CI], number of experiments), except where otherwise noted. Data without CI are single determinations.

(b) (4)

b This assay evaluated β5 activity.

c After exposure to 1-μM ixazomib for 30 minutes.

d Results are presented as the mean (95% CI, number of experiments).

(Excerpted from the submission)

Ixazomib induces apoptosis in multiple myeloma cells lines
Study no: MLN9708-27528,[1].

Methods

The effects of ixazomib on cell viability were studied using MM.1S, ANBL-6, RPMI-8226, and NCI-H929 human myeloma cell lines. Ixazomib was incubated up to 72 hours in culture at 10 concentrations, using 3-fold serial dilutions in dimethyl sulfoxide (DMSO) (n=2). Viability was measured and LD_{50} values were calculated by assaying cellular adenosine 5′-triphosphate (ATP) with the (b) (4) Viability testing was also conducted using colorimetric MTT assay in human multiple myeloma cell lines MM.1S, MM.1R, RPMI-8226, OPM1, OPM2, NCI-H929, and INA-6. Cells were incubated for 48 hours in 6.25, 12.5, 25, or 50 nM ixazomib in culture. To examine the mechanism of apoptosis, both mitrochondria-dependent (intrinsic, caspase-9 dependent) and independent (extrinsic, caspase-8 dependent) pathways were explored using immunoblot analysis of whole cell extracts from NCI-H929 and MM.1S ixazomib treated cells. Casapse-3, Caspase-8, Caspase-9, poly ADP ribose polymerase (PARP) were detected at full length (FL) and in their cleaved form (CF). Actin was used as a positive loading control.

Results

[1] Chauhan D, Tian Z, Zhou B, Kuhn D, Orlowski R, Raje N, et al. In vitro and in vivo selective antitumor activity of a novel orally bioavailable proteasome inhibitor MLN9708 against multiple myeloma cells. Clin Cancer Res. 2011;17(16):5311-21

Ixazomib inhibited cell viability dose dependently in all cell lines tested using the [redacted] (b) (4) with concentrations causing cell death for 50% of cells in culture (LD_{50}) in the nanomolar range (Table 6). Similarly using the MTT assay, cells cultured with ixazomib showed a dose dependent decrease in cell viability upon exposure (Figure 1). At concentrations of 12.5 nM in culture, MM.1S, MM.1R, and NCI-H929 cells exhibited over a 50% loss in cell viability and over 90% loss in cell viability at 25 nM. OPM1 and OPM2 were not as sensitive and only showed 40-50% loss in cell viability at 25 nM in culture. Immunoblot analysis showed increase in cleavage of caspase-3, caspase-8, caspase-9, and PARP, indicating that ixazomib induced both intrinsic mitochondria-dependent (caspase-9) and extrinsic mitochondria-independent (caspase-8) apoptosis (Figures 2 and 3).

Reference ID: 3844453

Table 6. LD_{50} values for ixazomib treated human myeloma cell lines

Cell Line	Disease	n	LD_{50} Geometric Mean (μM)	SE
ANBL-6	Multiple myeloma	3	0.0243	0.0072
MM.1S	Multiple myeloma	5	0.0258	0.0360
RPMI-8226	Plasmacytoma; myeloma	11	0.0059	0.0017
NCI-H929	Plasmacytoma; myeloma	5	0.0149	0.0007

Source: Report MLN9708-27528.

LD_{50} = concentration causing lethality for 50% of cells; SE = standard error.

Note: Data from at least 3 and up to 11 independent determinations are displayed as the geometric mean, along with the associated SE.

(Excerpted from the submission)

Figure 1. Effect of ixazomib on the viability of human multiple myeloma cell lines

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(Excerpted from Chauhan et al.2011)

Figure 2. Ixazomib induces caspase-mediated cell death in NCI-H929 multiple myeloma cell line

(*Excerpted from* Chauhan et al.2011)

Figure 3. Ixazomib induces caspase-mediated cell death in (b) (4) multiple myeloma cell line

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CF = cleaved fragment; FL = full length; H929 = NCI-H929; MLN2238 = ixazomib.
Panels A and B: H929 and MM.1S cells were treated with ixazomib for 24 hours and harvested. Results show immunoblot analysis of whole-cell lysates.

(Excerpted from Chauhan et al.2011)

Ixazomib induces cytotoxicity in multiple myeloma cells ex vivo

Source: Chauhan et al.[2]

[2] Chauhan D, Tian Z, Zhou B, Kuhn D, Orlowski R, Raje N, et al. In vitro and in vivo selective antitumor activity of a novel orally bioavailable proteasome inhibitor MLN9708 against multiple myeloma cells. Clin Cancer Res. 2011;17(16):5311-21

Methods

Primary myeloma cells were purified by CD138+ selection from 6 multiple myeloma patients. These patients included subjects who had relapsed after previous treatment with bortezomib, lenalidomide, and/or dexamethasone. Cells were treated ex vivo for 24 or 48 hours in culture at concentrations of 12, 25, or 50 nM ixazomib. Viability was measured using cellular adenosine 5′-triphosphate (ATP) and assayed with the (b) (4)

Results

Patient samples of multiple myeloma cells cultured in ixazomib ex vivo also showed a dose dependent decrease upon exposure to ixazomib (Figure 4). Following a 24-hour treatment with 25-nM ixazomib, the 6 patient samples examined showed approximately a 40% loss in cell viability relative to % of control. At concentrations of 50 nM ixazomib there was at least 70% loss in viability in 4 of the 6 patient samples.

Figure 4. Effect of ixazomib on the viability of human multiple myeloma patient cells ex-vivo

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(Excerpted from the Chauhan et al*)*

Ixazomib in combination with lenalidomide in vitro
Study no: MLN9708-30663

Methods
MM.1S, ANBL-6, RPMI-8226, and NCI-H929 were cultured in suspension for 120 hours in ixazomib, lenalidomide or a combination of the two up to 25 μM maximum concentrations. Culture plating densities were determined to ensure optimal linear growth over the experimental period. Cell proliferation was used as a measure of viability and was determined following lysis by assaying cellular adenosine 5′-triphosphate (ATP) with the (b) (4) Isobolograms were constructed to visualize the effect of combining ixazomib and lenalidomide treatments. The combination index (based on the isobologram) and non-linear blending values (see Peterson and Novick, J Recept Signal Transduct Res 2007; 27:125-146) were used to determine treatment synergy (additivity to antagonism).

Results
Ixazomib was cytotoxic in all multiple myeloma cell lines. Lenalidomide was cytotoxic to ANBL-6, NCI-H929, and RPMI-8226 cell lines and had no effect on cell viability in MM1.S cells at concentrations up to 25 μM. In combination, ixazomib and lenalidomide were synergistic in ANBL-6 and NCI-H929 cells and additive in MM.1S and RPMI-8226 cells as determined by both combination index and non-linear blending.

Table 7. Ixazomib in combination with lenalidomide in vitro

Cell Line	Compound	Findings	Interpretation
MM1.S	Ixazomib	EC_{50}: 23.9 nM	Additive
	Lenalidomide	EC_{50}: > 24,900 nM	
	Ixazomib and Lenalidomide	Combination index: N/A Nonlinear blending: 0	
ANBL-6	Ixazomib	EC_{50}: 17.7 nM	Synergistic
	Lenalidomide	EC_{50}: 578 nM	
	Ixazomib and Lenalidomide	Combination index: 0.45, $p < 0.001$ Nonlinear blending: 21.3, $p < 0.001$	
NCI-H929	Ixazomib	EC_{50}: 18.3 nM	Synergistic
	Lenalidomide	EC_{50}: 776 nM	
	Ixazomib and Lenalidomide	Combination index: 0.48, $p < 0.001$ Nonlinear blending: 23.6, $p < 0.001$	
RPMI-8226	Ixazomib	EC_{50}: 7.85 nM	Additive
	Lenalidomide	EC_{50}: 447 nM	
	Ixazomib and Lenalidomide	Combination index: N/A Nonlinear blending: 18.9, $p < 0.001$	

Source: Report MLN9708-30663.
EC_{50} = concentration producing a half-maximal response; N/A = not applicable.
Note: For combination index: when $p < 0.05$, combination index is defined as follows: synergy (CI 0-0.7); additivity (CI 0.7-1.3); subadditivity (CI 1.3 - 2); antagonism (CI > 2). If a conclusion cannot be made using combination index (CI), the nonlinear blending value (NLB) is used. When $p < 0.05$, NLB is defined as follows: synergy (NLB > 20); additivity (NLB between −20 and 20); antagonism (NLB < −20). When $p > 0.05$ for CI or NLB, the result is scored as inconclusive.

(Excerpted from the submission)

Pharmacokinetics and pharmacodynamics of single oral or intravenous administration of ixazomib in a mouse model of multiple myeloma
Study no: MLN9708-24699

Methods
Female CB-17 SCID mice with subcutaneously (SC) implanted MM.1S xenografts were treated by oral gavage (PO) or intravenously (IV) with ixazomib to determine antitumor activity. The vehicle used was 5% hydroxypropyl-beta-cyclodextrin [HP-β-CD], and single a single IV dose of ixazomib at 2.0 mg/kg, or a single PO of ixazomib at 6.0 mg/kg. Groups of 3 mice were examined and euthanized at 4 hours following the vehicle control group, and 0.25, 0.5, 1, 4, 8, or 24 hours following the ixazomib treated groups for pharmacokinetic analysis, and pharmacodynamic analysis by 20S proteasome β5 site-activity assay, immunoblotting for growth arrest and DNA damage-inducible protein (GADD34) and caspase-3, markers of cell death.

Results
Intravenous administration of 2-mg/kg ixazomib and oral administration of 6-mg/kg ixazomib resulted in similar plasma area under the concentration-time curve from the time 0 to 24 hours (AUC_{24}) (1100 and 1680 hr*ng/mL, respectively) (Table 8). These doses also showed similar tumor AUC_{24} for intravenous administration of 2-mg/kg ixazomib and oral administration of 6-mg/kg ixazomib (15,800 and 16,100 hr*ng/mL, respectively). One hour following administration of ixazomib, inhibition of the 20S proteasome β5 site, was observed and persisted up to 24 hours (Figure 5). The mean observed E_{max} for 20S proteasome inhibition in tumor tissue was 71.7% inhibition at the four hour time point and 60.6 % inhibition at 8 hours following IV and PO administration, respectively. GADD34 levels as detected by immunoblotting were increased 4 hours following ixazomib administration and remained elevated through 24 hours (Figure 6). By 8 hours following administration, cleaved caspase-3, a marker for apoptosis, was elevated and remained up to 24 hours postdose in both IV and PO samples (Figure 6).

Table 8. Summary pharmacokinetic parameters in human xenograft mouse model of multiple myeloma

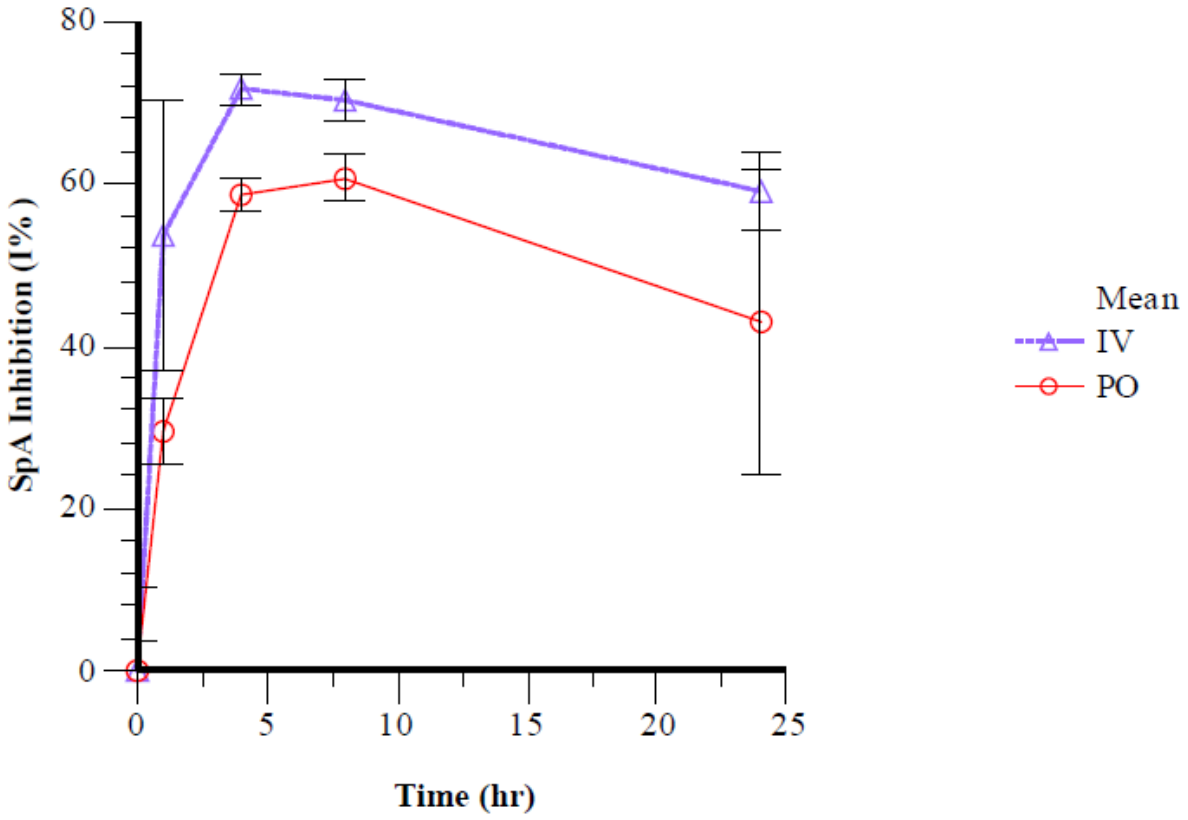
Route, Dose	Tissue	t_{max} (hr)	C_{max} (ng/mL)		AUC_{24} (hr*ng/mL)		Tumor-to-Plasma AUC Ratio
			Mean	SE	Mean	SE	
IV, 2 mg/kg	Plasma	0.25	1290	182	1100	48.2	N/A
	Tumor	1.0	902	40.7	15,800	798	14.4
PO, 6 mg/kg	Plasma	0.25	1540	379	1680	134	N/A
	Tumor	4.0	807	48.3	16,100	834	9.6

AUC = area under the concentration-time curve; AUC_{24} = area under the concentration-time curve from the time 0 to 24 hours; C_{max} = maximum observed concentration; IV = intravenous(ly); N/A = not applicable; PO = oral(ly); SE = standard error; t_{max} = time to reach C_{max}.

(Excerpted from the submission)

Reference ID: 3844453

Figure 5. Proteasome inhibition following treatment with ixazomib (IV, PO) in xenograft mouse model of multiple myeloma

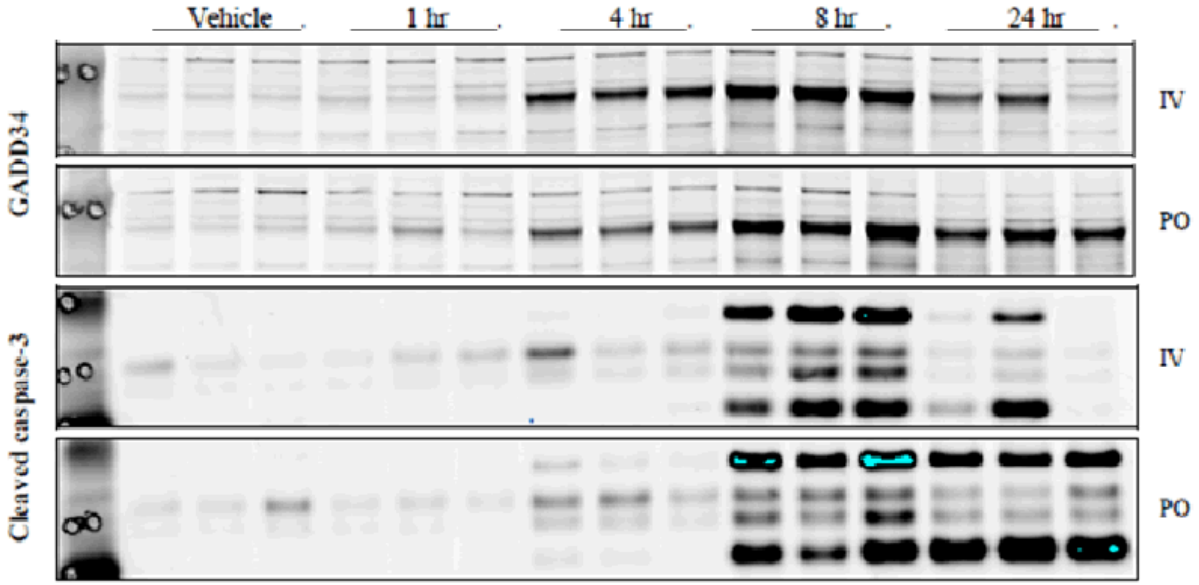


I% = percentage of proteasome inhibition; IV = intravenous(ly); PO = oral(ly); SpA = specific activity.

Vertical lines represent the standard deviation (SD); n = 3.

(Excerpted from the submission)

Figure 6. Immunoblotting analysis following treatment with ixazomib (IV, PO) in xeonograft mouse model of multiple myeloma



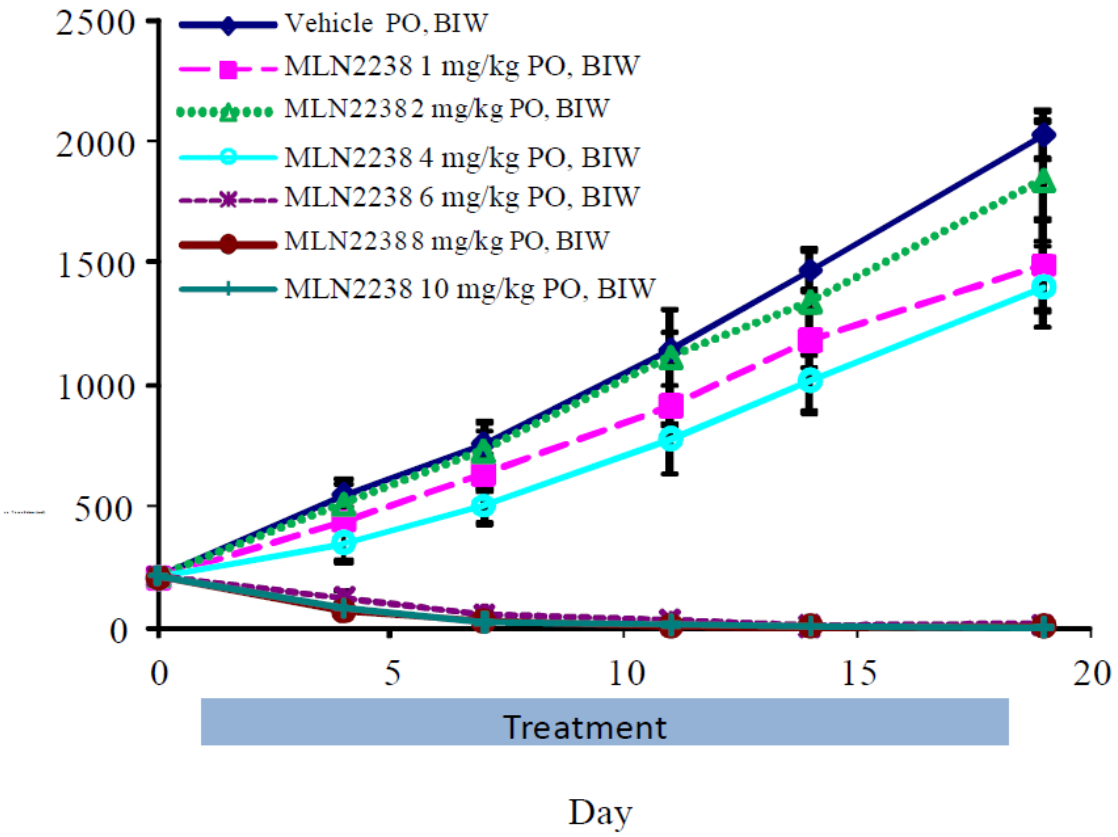
(Excerpted from the submission)

In vivo activity of ixazomib in a mouse model of multiple myeloma
Study no: MLN9708-24176

Methods
Using a mouse model of human multiple myeloma xenograft cells (MM.1S) in female CB-17 SCID mice vehicle (5% HP-β-CD) or ixazomib at 1, 2, 4, 6, 8, or 10 mg/kg was administered orally (PO) twice weekly for 18 days; a total of 5 doses. Day 1, mean tumor volumes (MTVs) measured approximately 100 to 350 mm^3. On Day 19, tumor growth inhibition (TGI) was calculated by using the percent TGI ([MTV of the control group - MTV of a treated group]*100 / [MTV of the control group]). Change in AUC was calculated by the change in the area under the tumor volume-versus-time curve, p values were calculated using a linear mixed effects regression model, with $p < 0.05$ considered statistically significant.

Results
Following treatment with ixazomib at 6, 8, and 10 mg/kg, there was a statistically significant decrease in mean tumor volume (Y-axis, mm^3) compared to vehicle control (TGI = 99.3%, 99.7%, and 100%, respectively). At 10 mg/kg, there were no measurable tumors on Day 19. At 6 and 8 mg/kg, tumor volume at Day 19 was less than that on Day 0. Changes in antitumor activity were statistically significant at both the 1 mg/kg and 4 mg/kg dose groups (TGI = 26.3% and 30.8%, respectively; change in AUC, $p > 0.005$ for both dosesbut not at the 2 mg/kg dose group. (TGI = 9.1%).

Figure 7. Mean tumor volume (mm^3) over time following treatment with ixazomib (biweekly, PO) in a human xenograft mouse model of multiple myeloma



BIW = twice weekly; MLN2238 = ixazomib; PO = oral(ly); SCID = severe combined immunodeficient.

Treatment began on Day 1. Error bars represent the standard error of the mean (SEM); n = 8 in the vehicle group and 5 in each treatment group.

(Excerpted from the submission)

4.2 Secondary Pharmacology

Studies not conducted.

4.3 Safety Pharmacology

Cardiovascular (GLP):

hERG patch-clamp HEK293 cells:

The effect of MLN9708 (100, 33.3, 11.1, 3.70, 1.23, 0.412, 0.137, 0.0457, 0.0152, and 0.00508 μM) on in vitro hERG current was evaluated in study RPT-04111 to assess the potential for delayed repolarization and prolongation of the QT interval. HERG (human-Ether-a-go-go Related Gene) is a gene encoding the pore forming subunit of a human delayed rectifying potassium channel, and blockade of hERG current has been associated clinically with delayed repolarization and proarrythmic responses in humans.

Binding assay was performed in a 96 well plate. Competition assays were performed using the ligand ^{3}H-dofetilide at 3.5nM (50% occupancy of receptor sites). The drug was added at a range of concentrations. Concentrations of 100, 33.3, 11.1, 3.70, 1.23, 0.412, 0.137, 0.0457, 0.0152, and 0.00508 µM MLN9708 were chosen to determine concentration response. Assays were initiated by adding membrane homogenates. Assay plates were placed on a shaking platform and incubated at room temperature for 80 minutes. The assay reaction was terminated by vacuum filtration onto polyethylenimine (PEI) treated glass fiber/barex (GF/B)-grade filters.

Results

- The IC_{50} for inhibition due to ixazomib was 59.6 µM, Ki value of 24.9 µM.
- The positive control, terfenadine, inhibited hERG potassium current with an IC_{50} value of 0.09 µM, mean Ki value of 0.04 µM.

Telemetry study in Dogs/Beagle for oral administration (GLP):
The effect of MLN9708 on cardiovascular parameters were assessed in telemetered dogs (n=4 males) in study B130356. Dogs were given single oral dose of vehicle or MLN9708 at 0.03, 0.2, and 0.3 mg/kg (0.021, 0.14, and 0.21 mg/kg as MLN2238; the biologically active form of MLN9708). The vehicle was 0.5 w/v% methylcellulose solution. Cardiovascular parameters included arterial blood pressures, pulse pressure, heart rate and electrocardiogram measurements 30 minutes prior to dosing and following dosing at 30 minutes 1, 2, 4, 8, and 24 hours. Parameters at different timepoints were compared to the animal's own baseline values.

Results

- There were no significant clinical signs.
- No affects were observed on any cardiovascular parameters.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Toxicokinetic assessments were included in the toxicology reports and are discussed in the toxicology section of this review.

Reference ID: 3844453

Table 9. Summary pharmacokinetics in nonclinical testing species following single dose oral administration of ixazomib or ixazomib citrate

Summary of Ixazomib Whole Blood Pharmacokinetic Parameters After a Single Dose of Ixazomib or Ixazomib Citrate												
Species	Dose (mg/kg)	Route	t_{max} (hr)	C_{max} (ng/mL)	AUC_{24} (hr*ng/mL)	AUC_t[a] (hr*ng/mL)	F[b] (%)	CL (mL/hr/kg)	$t_{1/2}$ (hr)	V_{ss} (mL/kg)	Formulation	Report[c]
Sprague-Dawley rats	0.8[d]	PO	0.83 ± 0.26	174 ± 24.4	2680 ± 172	N/A	34[e]	N/A	30 ± 5.1	N/A	Ixazomib in 10% HP-β-CD[f]	RPT-01124
	0.2[g]	IV	N/A[h]	200 ± 24.7	2270 ± 207	5580 ± 801 (96 hr)	N/A	26.3 ± 3.4	49 ± 4.0	1900 ± 248		RPT-01158
	0.3[g]	IV	N/A[h]	383 ± 86.5	2970 ± 269	N/A	N/A	44.1 ± 9.46	31 ± 7.1	1850 ± 179		RPT-01124
	0.4[g]	IV	N/A[h]	456 ± 81.6	2930 ± 116	6900 ± 2280 (96 hr)	N/A	50.0 ± 14.9	47 ± 22	2780 ± 210		RPT-01158
	0.6[i]	IPV infusion	0.50 ± 0.0	466 ± 64.1	3340 ± 483	N/A	N/A	N/A	24 ± 9.8	N/A		RPT-01124
New Zealand white rabbits	0.2[j]	PO	32.0 ± 13.9	24.2 ± 1.07	NC	1330 ± 52.5 (72 hr)	20.0	N/A	N/A	N/A	Ixazomib citrate in 55-mM citrate buffer and 3% glycine, pH 5.8[k]	MLN9708-24761-001A
	0.2[j]	IV	N/A[h]	351 ± 73.4	NC	6630 ± 794 (72 hr)	N/A	11.7 ± 2.63	65.4 ± 11.6	1050 ± 56.1		
Beagle dogs	0.4[l,i]	PO	0.67 ± 0.29	505 ± 43.5	7480 ± 511	17,900 (72 hr)[m]	105	N/A	> 72	N/A	Ixazomib in 10% HP-β-CD/ 2.8% mannitol in deionized water[n]	RPT-01106 Amend 1
	0.2[i]	IV infusion	N/A[h]	609 ± 48.4	3570 ± 36.1	8740 ± 509 (72 hr)	N/A	8.70 ± 2.72	> 72	1260 ± 80		RPT-01106 Amend 1
Cynomolgus monkeys	0.1[i]	IV infusion	N/A[h]	297 ± 48.2	1410 ± 100	3130 ± 175 (72 hr)	N/A	18.3 ± 1.62	57.5 ± 5.1	1520 ± 82	Ixazomib in 10% HP-β-CD in deionized water	RPT-01105

(Excerpted from the submission)

Absorption

Pharmacokinetics of MLN2238 after oral administration of MLN9708 capsules to non-naive male beagle dogs

Key study findings

- Absorption of MLN2238 was moderate following oral dosing (t_{max} 0.5 h).
- The mean plasma C_{max} value was 279 ng/mL for MLN2238 following oral dosing.
- The mean plasma $AUC_{(0\text{-}24h)}$ value for MLN2238 was 973 hr*ng/mL following oral dosing.

Study no.:	RPT-01218
Study report location:	eCTD 4.2.2.2
Conducting laboratory and location:	Millenium Pharmaceuticals Inc, Cambridge, MA, USA
Date of study initiation:	April 25, 2008
GLP compliance:	No
QA statement:	No
Drug, Batch #, and % purity:	MLN9708, Batch 8620-02, 99%

Methods

Liquid chromatography with tandem mass spectrophotometry (LC/MS/MS)

Species/strain:	Beagle dogs
N:	4 males
Dose:	1.48 mg
Frequency:	single dose
Route:	oral capsule (powder-in-capsule formulation)

Reference ID: 3844453

Volume: capsule given with 10 mL distilled water; 5 mL distilled water flush

Observations and times: Samples collected from a peripheral vessel at 0, 15, 30 minutes and 1, 2, 4, 6, 8, and 24 hr post dose. The lower limit of quantitation (LLOQ) was 1.00 ng/mL.

Results

Table 10. Pharmacokinetic parameters of MLN2238 following oral dosing in dogs

	Dose (mg/kg)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-24hr} (hr*ng/mL)
Mean[a]	0.145	1.0	279	973
SD	0.0212	0.7	149	241
%CV	N/A	70.7	53.3	24.7

%CV = percent coefficient of variation; AUC_{0-24hr} = area under the concentration-versus-time curve 0 to 24 hours; C_{max} = maximum concentration; hr = hour(s); SD = standard deviation; T_{max} = time to maximum concentration.

a n = 4.

(Excerpted from the submission)

Table 11. Mean Plasma concentrations of MLN2238 in beagle dogs following a single oral dose

Time Point (hr)	ML2238 (ng/mL)	
	Mean[a]	SD
0	BQL[b]	N/A[c]
0.25	64.0	54.1
0.5	195	224
1	193	71.4
2	114	33.7
4	96.5	31.6
6	26.7	23.9
8	24.6	24.9
24	13.0	5.25

BQL = below the quantitation limit; hr = hour(s); N/A = not applicable; SD = standard deviation.

a n = 4, unless specified otherwise.

b <1.00 ng/mL.

c All samples BQL.

(Excerpted from the submission)

Distribution

Tissue Distribution via Quantitative Whole-Body Autoradiography in Male Long-Evans Rats Following a Single Oral Gavage Administration of [^{14}C] MLN9708 and Human Radiation Dosimetry Estimation

Key study findings

- Tissue half-life for radioactivity was longest in the urinary bladder ($t_{1/2}$= 946 h) and sciatic nerve (887 h). In addition, the highest Cmax of radioactivity was observed in the urinary bladder.
- In addition to these organs, exposure based on Cmax and/ or AUC was high in lymph node, spleen, adrenal glands, kidney (renal cortex and medulla), bile colon, small intestine, cecum and liver, consistent with renal and hepatobiliary routes of excretion.
- Relatively low amount of radioactivity in the brain indicates that drug-related materials don't likely cross the blood-brain barrier.

Study no.:	96N-1206
Study report location:	eCTD 4.2.2.2
Conducting laboratory and location:	Millenium Pharmaceuticals Inc, Cambridge, MA, USA
Date of study initiation:	April 1, 2012
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[^{14}C] MLN9708, Lot # MP-E2763-1, > 95%; MLN9708, Lot # 100895, 99.7%

Methods

Tissue levels of [^{14}C] radioactivity detected by whole body radiography ranged from 0.003 to 4.412 µg equiv/g. [^{14}C] MLN9708 and MLN9708 were formulated in 55mM citrate, 1% propylene glycol, pH 5.2. The concentration of radioactivity of [^{14}C] MLN9708 in formulation was 13.11 µCi/g and the specific activity of the [^{14}C] MLN9708 in formulation was 112.25 µCi/mg.

Species/strain:	Long Evans (n=9) pigmented rats
N:	9 male
Dose:	0.6 mg/kg (approximately 65 µCi/kg)
Frequency:	single dose
Route:	oral gavage
Volume:	5 ml/kg

Observations and times: 0.5, 1, 4, 12, 24, 72, 168, 336 and 672h post-dose

Reference ID: 3844453

Table 12. Study design for tissue radioactivity analysis of Long Evans rats following a single oral dose of MLN9708

Group	Number/ Strain/ Sex	Dose Route	Dose Formulation[a]	Target Dose Level (mg/kg)	Target Dose Volume (mL/kg)	Target Dose Concentration (mg/mL)	Target Radioactivity Level (µCi/kg)
1	9/ Long-Evans/ Male	PO	Solution	0.6	5	0.12	~ 65

PO = *per os* (oral administration).

[a] Dose formulation was prepared by mixing [redacted] (b) (4) [redacted] (b) (4)

(Excerpted from the submission)

Results

Table 13. Pharmacokinetic parameters of tissue radioactivity following single oral dose of MLN9708 in male Long Evans rats

Organ/Tissue	C_{max} (µg equiv/g)	$t_{1/2}$ (h)	No. of points in $t_{1/2}$	r^2	T_{max} (h)	AUC_{inf_obs} (µg equiv•h/g)	AUC (µCi•h/g)	Drat (rem)
Adipose (brown)	0.4500	212.8	3	0.87	1.0	23.3407	2.6200	0.275
Adipose (white)	0.0940	124.2	3	0.98	0.5	3.7221	0.4178	0.044
Adrenal Gland	2.6780	312.5	3	0.98	4.0	223.0532	25.0377	2.629
Blood (cardiac)	0.4390	165.1	5	0.87	0.5	28.6836	3.2197	0.338
Bone	0.0300	37.1	4	0.88	0.5	1.1261	0.1264	0.013
Bone Marrow	0.7420	149.4	5	0.90	1.0	62.5787	7.0245	0.738
Brain (cerebellum) a	0.0190	263.5	6	0.08	0.5	3.2799	0.3682	0.039
Brain (cerebrum) a	0.0130	276.1	7	0.07	0.5	3.5814	0.4020	0.042
Brain (medulla) a	0.0130	586.4	6	0.02	0.5	6.4684	0.7261	0.076
Cecum	2.8040	153.9	4	0.96	4.0	104.7165	11.7544	1.234
Dorsal Root Ganglion b	0.1260	86.1	4	0.97	0.5	11.2376	1.2860	0.135
Epididymis a	0.1480	813.6	5	0.62	4.0	106.5211	11.9570	1.255
Esophagus b	0.4860	86.3	4	0.96	0.5	14.6372	1.6723	0.176
Eye (lens) a, b	0.0050	ND	0	ND	4.0	ND	0.0759	0.008
Eye (uveal tract) a	0.1870	236.3	5	0.68	1.0	17.6522	1.9815	0.208
Harderian Gland	0.3540	155.9	4	0.89	4.0	38.3394	4.3036	0.452
Heart (myocardium)	0.6020	180.2	4	1.00	0.5	30.5114	3.4249	0.360
Large Intestine b	1.7780	165.9	4	0.87	12.0	68.7226	7.7141	0.810
Liver	3.1150	197.7	4	1.00	0.5	129.3783	14.5227	1.525
Lung	0.4330	235.4	4	0.94	0.5	59.4325	6.6713	0.700
Lymph Node	0.5300	289.7	3	0.85	168.0	270.7915	30.3963	3.192

(Excerpted from the submission)

Organ/Tissue	C_{max} (µg equiv/g)	$t_{1/2}$ (h)	No. of points in $t_{1/2}$	r^2	T_{max} (h)	AUC_{inf_obs} (µg equiv•h/g)	AUC (µCi•h/g)	Drat (rem)
Mammary Gland Region	0.1150	252.5	4	0.94	1.0	20.6064	2.3131	0.243
Oral Mucosa	0.2640	187.7	4	0.99	1.0	24.6872	2.7711	0.291
Pancreas	0.6890	163.2	4	0.91	0.5	32.1879	3.6131	0.379
Pituitary Gland	0.7570	236.7	4	0.98	4.0	120.4020	13.5151	1.419
Prostate Gland	0.1640	229.9	4	0.93	1.0	31.6779	3.5558	0.373
Renal Cortex	2.3970	145.8	5	0.85	0.5	70.2350	7.8839	0.828
Renal Medulla	1.5770	175.4	4	0.86	0.5	70.8088	7.9483	0.835
Salivary Gland	0.9300	212.4	4	0.90	0.5	66.8660	7.5057	0.788
Sciatic Nerve a	0.1460	887.0	4	0.69	0.5	10.9484	1.2290	0.129
Seminal Vesicles a	0.1090	325.3	4	0.84	4.0	9.7231	1.0914	0.115
Skeletal Muscle	0.2000	173.0	4	0.87	1.0	12.8868	1.4465	0.152
Skin (non-pigmented)	0.1470	158.0	4	0.94	1.0	17.3099	1.9430	0.204
Skin (pigmented)	0.1690	201.8	4	0.91	1.0	22.6171	2.5388	0.267
Small Intestine [a]	4.3840	194.3	4	0.77	0.5	136.5487	15.3276	1.609
Spinal Cord [a]	0.0150	201.2	6	0.18	0.5	2.3018	0.2584	0.027
Spleen	1.1850	332.3	4	0.92	0.5	308.3645	34.6139	3.634
Stomach (gastric mucosa)	1.1620	167.5	4	0.90	0.5	46.6681	5.2385	0.550
Testis [a]	0.0590	765.8	5	0.74	4.0	48.6781	5.4641	0.574
Thymus	0.2900	262.9	3	0.85	168.0	132.5326	14.8768	1.562
Thyroid	0.9020	172.0	4	0.94	0.5	67.7353	7.6033	0.798
Urinary Bladder	4.412	946.1	3	1.00	4.0	82.6822	9.2811	0.975

ND = Not determined; unable to determine value from the data

a = $t_{1/2}$ values for these tissue/organs are obtained from insufficient data points and/or the resulting r^2 values fall outside acceptance criteria (b) (4) thus, these values should be considered as estimate.

b = Unable to determine AUCinf_obs or AUCall was higher than AUCobs_inf, so AUCall used for prediction.

c = Tissue with highest exposure before weighting

D= absorbed dose in dosimetry prediction

(Excerpted from the submission)

Metabolism

Pharmacokinetics, Metabolism, Mass Balance, and Excretion Routes of [^{14}C] MLN9708 In Male Sprague-Dawley Rats Following A Single PO Dose

Key study findings

- Similar excretion patterns were observed in bile duct cannulated (BDC) and intact rats following a single oral dose (0.8 mg/kg). Mean total recovery was 80.7 % and 87.2% respectively.
- In BDC rats, mean urinary excretion of radioactivity over 72 hours was 23.0 % of the dose.
- In BDC rats, mean biliary and fecal excretion accounted for 22.3 % and 35.4 % of the dose, respectively.
- A higher percentage of excretion of radioactivity into feces unabsorbed compound following oral administration.

Study no.: MLNR1800R2
Study report location: eCTD 4.2.2.2
Conducting laboratory and Location: Millenium Pharmaceuticals Inc, Cambridge, MA, USA
Date of study initiation: January 26, 2012
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: See Table 14

Methods

Species/strain: Han Wistar rats
N: 31 total; See Table 15 and 16 for study design, sample collection
Dose: 0.8 mg/kg
Frequency: single dose
Route: oral gavage
Volume: 4 ml/kg
Observations and times: See Table 16

Table 14. Test articles used in a pharmacokinetic study of metabolism, mass balance and excretion in rats following oral administration

Compound	Lot Number	MW	SA	Purity (b) (4)
MLN9708-001	100895	517.12	NA	99.7%
MLN2238-004	115001	361	NA	99.9%

(Excerpted from the submission)

Table 15. Study design of metabolism, mass balance and excretion study in rats following single oral administration

Group No.	Dosing Route	Surgical Model	No. of male rats	Target Dose Information			
				(mg/kg)	(µCi/kg)	(mg/mL)	(mL/kg)
1	PO	Intact	3	0.8	88	0.2	4
2		JVC / BDC	4				
3		Intact	24				

(Excerpted from the submission)

Reference ID: 3844453

Table 16. Sample collection in metabolism, mass balance and excretion study in rats following single oral administration

Group No.	Metabolism and Pharmacokinetics				
	Blood	Bile	Urine	Feces	Cage Wash/Wipe
1 (N = 3; Intact)	N/A	N/A	Pre-dose, 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168, 168-192, 192-216, and 216-240h post-dose	Pre-dose, 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168, 168-192, 192-216, and 216-240h post-dose	240 h Post-dose
2 (N = 4; BDC/JVC)	Pre-dose, 0.5, 1, 2, 4, 6, 8, 24h post-dose (350µL/time-point)	Pre-dose, 0-6, 6-24, 24-48, and 48-72h post dose	Pre-dose, 0-6, 6-24, 24-48, and 48-72h post-dose	Pre-dose, 0-24, 24-48, and 48-72h post-dose	72h post-dose
3 (N=24, intact)	Pre-dose, 0.5, 1, 2, 4, 6, 8, 24h post-dose (N = 3/ time point, terminal collection)	N/A	N/A	N/A	N/A
Volume/ time-point	See Above	Total Collected	Total Voided		N/A
Anticoagulant	K_2EDTA	N/A			

No. = Number; N/A = Not Applicable
Note: For Group 2, 250 µL of pooled blood samples were removed prior to centrifugation for each plasma sample; For Group 3, 400 µL of blood samples were removed prior to centrifugation for plasma samples.

(Excerpted from the submission)

Results

Table 17. Mean percent excretion of radioactivity in urine and feces in intact rats following a single oral dose of [^{14}C]MLN9708

Parameters	% Dose (0-24 hr) (n = 3)	% Dose (0-72 hr) (n = 3)	% Dose (0-240 hr) (n = 3)
Urine	14.14 ± 6.51	20.75 ± 8.74	26.98 ± 13.22[a]
Feces	24.10 ± 13.32	53.03 ± 15.08	60.22 ± 13.03
Total	38.23 ± 11.86	73.78 ± 6.34	87.20 ± 1.19[a]

a Includes cage wash at 240 hr post-dosing.

(Excerpted from the submission)

Table 18. Mean percent excretion of radioactivity in urine and feces in bile duct cannulated rats following a single oral dose of [^{14}C] MLN9708

Parameters	% Dose (0-24 hr) (n = 3)[a]	% Dose (0-48 hr) (n = 3)[a]	% Dose (0-72 hr) (n = 3)[a]
Urine	9.44 ± 2.52	15.97 ± 2.88	22.96 ± 3.73[a]
Bile	17.27 ± 10.15	20.46 ± 9.44	22.33 ± 8.43
Feces	7.72 ± 6.33	24.35 ± 0.87	35.41 ± 8.41
Total	34.42 ± 6.93	60.78 ± 11.46	80.70 ± 4.90[b]

a Urine, bile and fecal samples were obtained from Rat #4 (study MIL-P1800) and from Rat #A and #B (study MIL-P1862) to determine recovery of radioactivity over 3 days. Rats 1-3 from study MIL-P1800 were not used to obtain the mean cumulative excretion values since they expired on Day 2.

b Includes cage wash at 72 hr post-dosing.

6 General Toxicology

6.1 Single-Dose Toxicity

Studies not reviewed.

6.2 Repeat-Dose Toxicity

Study title: A 6-month Oral Gavage Toxicity Study of MLN9708 with a 2-Week Recovery Period in Sprague Dawley Rats

The following study was initially reviewed under IND (b) (4) by Matt Whittaker PhD and parts of it were reformatted to fit this NDA review.

Study no.:	(b) (4) 416165
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 3, 2013
GLP compliance:	Signed and Included
QA statement:	Signed and Included
Drug, lot #, and % purity:	MLN9708, Lot # 101296; 99.1%

Key Study Findings

- There were 5 early mortalities in the high dose group (0.6/0.8 mg/kg) and 2 mortalities in the mid dose group (0.4 mg/kg). The cause of death in the high dose group was attributed to toxicities in the intestine, liver, and/or lymphoid depletion. Mortalities at 0.4 mg/kg were attributed to renal failure (non-test article related).
- Clinical signs include decreased defecation, soft feces, small feces, yellow material around the urogenital and anogenital areas and forelimbs, hypoactivity, thin appearance, diarrhea, and clear discharge from the eyes.

Reference ID: 3844453

- Toxicologically significant ixazomib hematology findings included higher mean values of absolute counts for total white blood cells, neutrophils, lymphocytes, and monocytes.
- Histopathology findings included neutrophilic infiltrates in the lamina propria, epithelial hyperplasia and necrosis, villous atrophy in the gastrointestinal tract; hepatocellular degeneration/necrosis, hepatocellular vacuolation, and neutrophil infiltration in the sinusoids; lymphoid depletion and necrosis in lymph nodes and spleen.

Methods

Doses:	0, 0.2, 0.4, 0.6/0.8 mg/kg/week
Frequency of dosing:	7 x 28 day cycles; dosing on days 0, 7, and 14, followed by a 13-day non-dosing period.
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	55 mM citrate buffer, 0.45% NaCl, pH 5.8
Species/Strain:	Adult male and female Sprague-Dawley rats
Number/Sex/Group:	15/sex/group
Age:	7 to 8 weeks
Weight:	230 to 280 g (males) or 168 to 230 g (females)
Satellite groups:	N/A
Unique study design:	N/A
Deviation from study protocol:	None impacted study results or objectives

Table 19. Study design for 6 month repeat dose toxicology study in rats

Group	Control or Test Article	Dosage Level (mg/kg/dose)[a]	Dose Volume (mL/kg)	Number of Animals[b]	
				Males	Females
1	Vehicle	0	5	15	15
2	MLN9708	0.2	5	15	15
3	MLN9708	0.4	5	15	15
4	MLN9708	0.8/0.6[c]	5	15	15

a Ten (10) animals per sex per group were euthanized at the primary necropsy (Day 183); the remaining ≤ 5 animals per sex per group were euthanized after a 14-day nondosing (recovery) period.

b MLN9708 formulations were adjusted by a factor of [redacted] (b) (4) to account for the citrate ester, purity, and content.

c Starting on Day 56 (first dose of third cycle), the Group 4 female dosage level was lowered to 0.6 mg/kg/dose (dose concentration of 0.12 mg/mL, dose volume of 5 mL/kg).

(Excerpted from the submission)

Observations and Results

Mortality

Table 20. Cause of mortality in 6 month repeat dose toxicology study in rat

Animal #	Sex	Dose (mg/kg)	Date of death	Last dose	Clinical signs	Body weight	Histopathology findings	Cause of death
1842	Male	0.4	Day 159	Day 154	Days 156 - 158 ▪ Red material around nose, eye, mouth, forelimbs, urogenital & anogenital area ▪ Red penile discharge ▪ Yellow material urogenital area	Wk 21 – 22: ▼ 23 g (wk prior to death)	*Kidney* ▪ Bilateral pyelonephritis ▪ Transitional cell (urothelial) hyperplasia – renal pelvis *Ureters* ▪ Calculi ▪ Chronic inflammation ▪ Transitional cell hyperplasia *Urinary bladder* ▪ Calculi, multiple (5 – 8 mm diameter) ▪ Chronic inflammation ▪ Transitional cell (urothelial) hyperplasia *Lung* ▪ Mineralization of alveolar septae *Heart* ▪ Myocardial degeneration ▪ Mineralization of aorta *Prostate* ▪ chronic active inflammation (mod) *Bone marrow* ▪ myeloid hyperplasia *Lymphoid tissues* ▪ Lymphoid depletion (min – mod)	▪ **Renal failure** secondary to urinary blockage from calculi (This death was judged to be unrelated to drug treatment).
1850	Male	0.4	Day 176	Day 175	Days 28 – 114 ▪ Yellow material around urogenital & anogenital areas, ventral trunk and hindlimbs	Wk 24– 25: ▼ 103 g (wk prior to death)	*Kidney* ▪ Bilateral pyelonephritis (marked) ▪ Dilated pelvis ▪ Calculi, bilateral ▪ Transitional cell (urothelial) hyperplasia – renal pelvis, *Ureters* ▪ Calculi ▪ Chronic inflammation	▪ Pelvic organ and kidney inflammation with **urinary calculi** (This death was judged to be unrelated to drug treatment).

Animal #	Sex	Dose (mg/kg)	Date of death	Last dose	Clinical signs	Body weight	Histopathology findings	Cause of death
							▪ Transitional cell hyperplasia *Urinary bladder* ▪ Calculi ▪ Transitional cell (urothelial) hyperplasia *Prostate* ▪ chronic active inflammation (mod) Seminal vesicles ▪ chronic active inflammation (mod) *Lymphoid tissues* ▪ Lymphoid depletion/necrosis (min – mod) *Heart* ▪ Myocardial degeneration, multifocal	
1908	Female	0.8	Day 9	Day 7	Day 8 ▪ Dried red material around nose ▪ Diarrhea	Wk 0 – 1: ▲ 17g	*Rectum* ▪ single cell necrosis, lamina propria ▪ epithelial hyperplasia, diffuse *Liver* ▪ Hepatocellular vacuolation (marked) ▪ Generalized hepatocellular degeneration/necrosis *Lymphoid tissue* ▪ Necrosis ▪ Lymphoid depletion *Bone marrow* ▪ Hypocellularity Single cell necrosis, multifocal *Lungs* ▪ Lymphoid necrosis ▪ BALT edema, multifocal ▪ Intravascular neutrophilia	▪ Liver and lymphoid lesions

Animal #	Sex	Dose (mg/kg)	Date of death	Last dose	Clinical signs	Body weight	Histopathology findings	Cause of death
1900	Female	0.8	Day 29	Day 28	Days 20- 29 ▪ Dried red material around nose ▪ Wet brown material, anogenital & urogenital area	Wk 3 – 4: ▲ 27g	*GI tract (duodenum, ileum, jejunum, cecum, rectum)* ▪ Neutrophil infiltrate ▪ Epithelial necrosis: lamina propria *Liver* ▪ Hepatocellular vacuolation (marked) ▪ Generalized hepatocellular degeneration/necrosis *Lymphoid tissue* ▪ Necrosis	▪ Liver, intestinal & lymphoid lesions
1869	Female	0.8	Day 29	Day 28	Day 29 ▪ Partial closure: L & R eyes	Wk 3 – 4: ▲ 11 g	*GI tract (duodenum, ileum, jejunum, cecum, rectum)* ▪ Neutrophil infiltrate ▪ Epithelial necrosis: crypts, lamina propria *Liver* ▪ Hepatocellular vacuolation (marked) ▪ Generalized hepatocellular degeneration/necrosis *Lymphoid tissue* ▪ Necrosis *Bone marrow* ▪ Hypocellularity ▪ Single cell necrosis, multifocal	▪ Liver, intestinal & lymphoid lesions
1888	Female	0.8	Day 36	Day 35	▪ No clinical signs immediately prior to death	Wk 4 - 5: ▲ 19 g	*GI tract (duodenum, ileum, jejunum, cecum, rectum)* ▪ Neutrophil infiltrate ▪ Epithelial necrosis: crypts, lamina propria ▪ Villous atrophy (flattening & fusion of the villi) *Liver* ▪ Hepatocellular vacuolation (marked) ▪ Generalized hepatocellular degeneration/necrosis *Lymphoid tissue* ▪ Necrosis *Bone marrow* ▪ Hypocellularity ▪ Single cell necrosis, multifocal *Heart* ▪ Hemorrhage, multifocal *Kidney* ▪ Tubular basophilia ▪ Mineralization, cortex, bilateral *Lungs* ▪ Mixed inflammatory cell infiltrate, perivascular ▪ Intravascular neutrophilia	▪ Liver, intestinal & lymphoid lesions
1891	Female	0.8/0.6	170	168	Days 1-157 ▪ Dried yellow material around urogenital area Days 2 – 143 ▪ Dried red material around mouth Days 1 – 114 ▪ Dried red material around nose Days 142 – 169 ▪ Decreased defecation, feces small, soft feces	Wk 23 - 24: ▲ 2 g	*Duodenum, ileum* ▪ Neutrophil infiltrate ▪ Epithelial necrosis: crypts *Liver* ▪ Hepatocellular vacuolation (marked) ▪ Generalized hepatocellular degeneration/necrosis *Lymphoid tissue* ▪ Necrosis	▪ Liver, intestinal & lymphoid lesions

Two mid-dose male rats (0.4 mg/kg) died prematurely due to causes related to calculi formation and renal failure. The incidence of calculi findings in the current study (2/15 = 13.3%) was within the range of historical control values in rats aged 7 – 32 weeks (same age range of rats in the current study). The submitted historical control data ((b) (4)

Research, studies conducted between 2007 - 2014) are summarized in the following table:

Table 21. Supplemental information: summary of historical control data pertaining to calculi formation in kidney and urinary bladder in male

Finding	Tissue	Male			Female		
		Total incidence (%)	Total # of studies / Total # of animals	Individual study incidence range (%)	Total incidence (%)	Total # of studies / Total # of animals	Individual study incidence range (%)
Calculi	Kidney	0.16% (2/1285)	275 / 1285	9.1 -11.1%	0.64% (11/1715)	281 / 1715	7.1 – 60.0%
	Urinary bladder	0.20% (9/4413)	376 / 4413	5.0 – 10.0%	0.16% (7/4332)	364 / 4332	6.7 – 20.0%

(Excerpted from the submission)

It is noted that while there was evidence for multiple sporadic kidney findings of minimal severity (e.g. tubular basophilia, mononuclear cell infiltrate, tubular mineralization) in animals in all dose groups, there were no dose-related renal pathologies observed in this study. Therefore, it appears that the kidney is not a target organ of toxicity for oral ixazomib in rats.

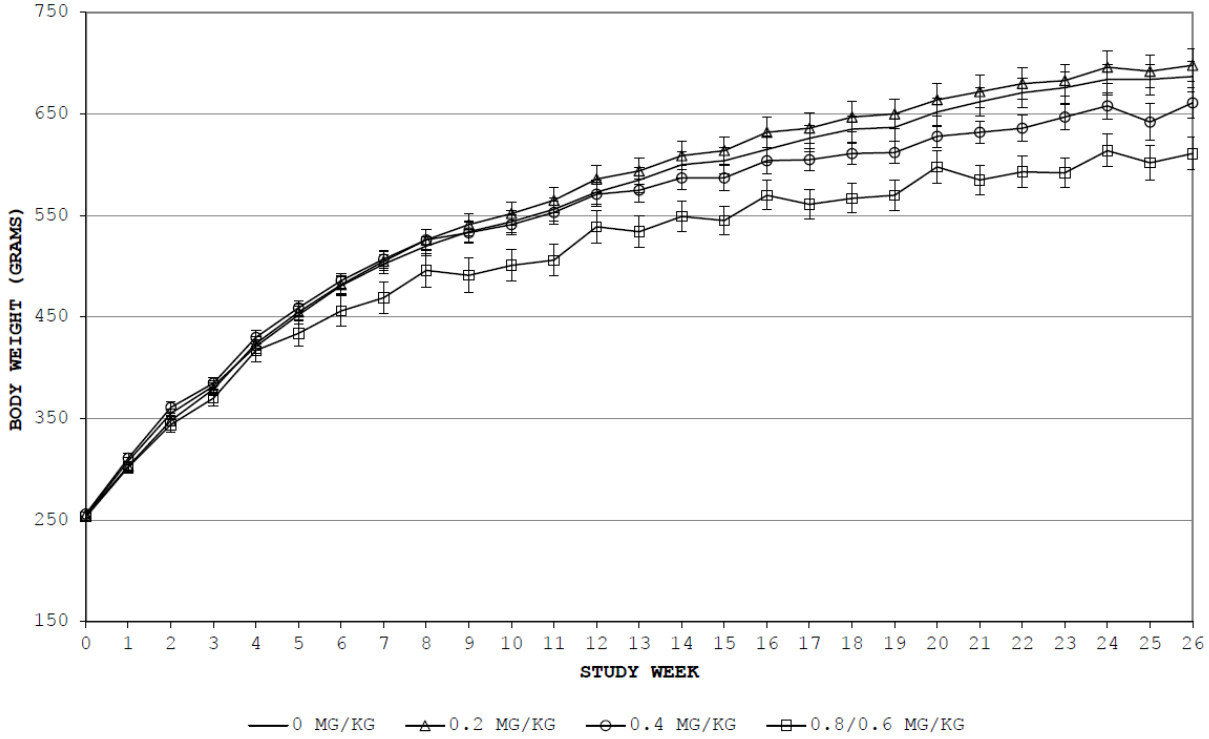
Clinical Signs

Animals in the 0.8/0.6 mg/kg exhibited decreased defecation, soft feces, small feces (females only), yellow material around the urogenital and anogenital areas and forelimbs, hypoactivity, thin appearance, diarrhea, and clear discharge from the eyes.

Body Weights

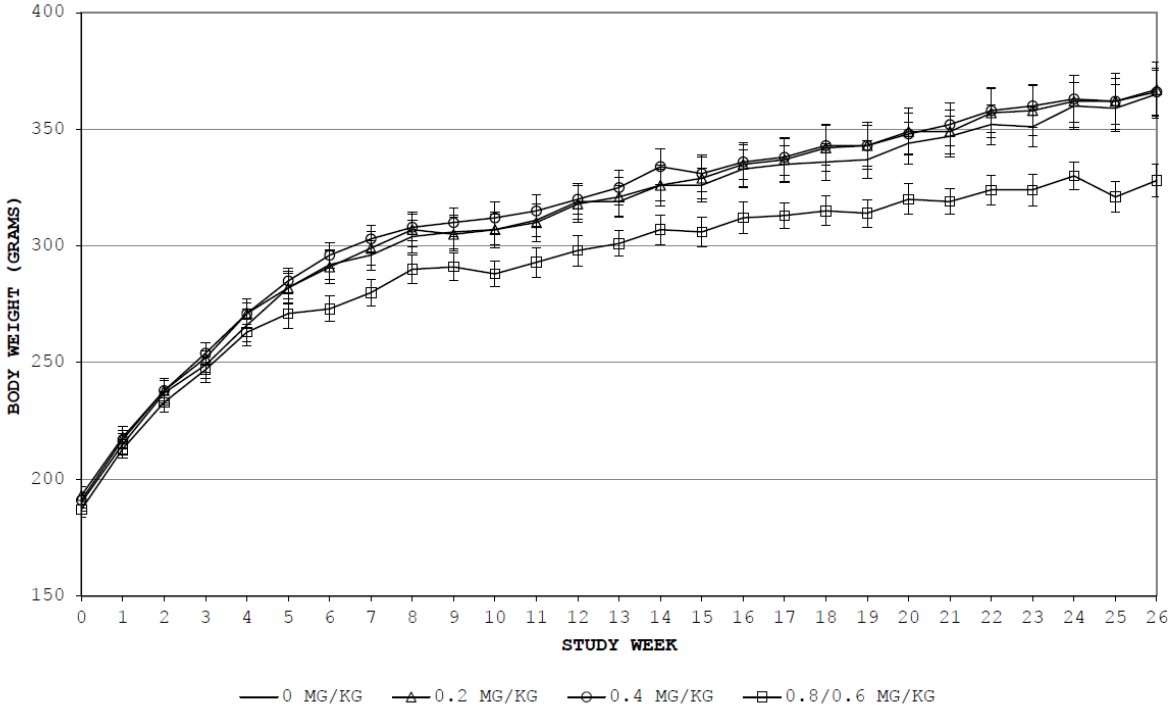
Statistically significant changes in body weight were observed in the 0.8/0.6 mg/kg dose group at 25 weeks. At Week 26, males and females had 11.1% and 10.1% lower mean body weight relative to controls, respectively.

Figure 8. Body weights in male rats in 6 month repeat dose toxicology study



(Excerpted from the submission)

Figure 9. Body weights in female rats in 6 month repeat dose toxicology study



(Excerpted from the submission)

Feed Consumption

Feed consumption was reduced in animals in the 0.8/0.6 mg/kg group males and females, beginning during Week 4 to 5 and persisted throughout the study.

Ophthalmoscopy

Visual examinations were not performed.

Hematology

Hematology and coagulation were collected on all animals at the scheduled necropsies (Weeks 26 and 28, main study and recovery period respectively).

Table 22. Hematology findings for 6 month repeat dose toxicity study in rats (percent change from control)

		Males (mg/kg)			Females (mg/kg)		
Parameter	**Interval (Week)**	**0.2**	**0.4**	**0.8**	**0.2**	**0.4**	**0.8/0.6**
WBC	26	n	105.3**	151.3**	n	54.5	77.2**
	28	n	n	n	n	n	NA
Abs. NEU	26	n	304.7**	530.2**	n	151.5	248.5*
	28	n	n	n	n	n	NA
Abs LYMPH	26	n	39.2*	23.6	n	29.9	36.6
	28	n	n	n	n	n	NA
Abs MONO	26	n	62.9*	97.1**	n	72.2*	44.4
	28	n	n	n	n	n	NA
Abs BASO	26	n	100.0	100.0**	n	n	n
	28	n	n	n	n	n	NA
RDW	26	n	n	n	n	n	n
	28	n	10.9*	16.3**	n	n	NA
APTT	26	n	n	n	n	n	10.2**
	28	n	n	n	n	n	NA

Abs = absolute; n = no noteworthy findings – or – no test article-related effect. NA – not applicable

(Excerpted from the submission)

WBC: white blood cells; NEU: neutrophils; LYMPH: lymphocytes; MONO: monocytes; RDW: red cell distribution width; APTT: activated partial thromboplastin time
* Significantly different from the control group at 0.05
** Significantly different from the control group at 0.01

Clinical Chemistry

Clinical chemistry parameters were collected on all animals at the scheduled necropsies (Weeks 26 and 28, main study and recovery period respectively). All clinical chemistry parameters were unremarkable.

Urinalysis

Urinalysis was not conducted

Gross Pathology

Unremarkable

Organ Weights

Unremarkable

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

Table 23. Histological findings from 6 month repeat dose toxicology study in rats

	Main study (day 183)								Recovery (Day 196)							
	Males				Females				Males				Females			
MLN 9708 dose (mg/kg)	0	0.2	0.4	0.8	0	0.2	0.4	0.8/0.6	0	0.2	0.4	0.8	0	0.2	0.4	0.8/0.6
number of animals in study	10	10	10	10	10	10	10	10	5	5	5	5	5	5	5	5
DUODENUM (# examined)	10	10	10	10	10	10	10	10	5	0	0	5	5	0	5	0
Epithelial hyperplasia																
minimal	0	5	3	1	0	6	5	4	0	-	-	0	0	-	0	-
mild	0	5	7	9	0	0	5	5	0	-	-	0	0	-	0	-
moderate	0	0	0	0	0	0	0	1	0	-	-	0	0	-	0	-
TOTAL	**0**	**10**	**10**	**10**	**0**	**6**	**10**	**10**	**0**	**-**	**-**	**0**	**0**	**-**	**0**	**-**
Infiltrate, neutrophil																
minimal	1	1	6	8	0	1	4	4	0	-	-	0	0	-	0	-
mild	0	0	2	2	0	0	0	0	0	-	-	0	0	-	0	-
TOTAL	**1**	**1**	**8**	**10**	**0**	**1**	**4**	**4**	**0**	**-**	**-**	**0**	**0**	**-**	**0**	**-**
Single cell necrosis																
minimal	0	1	2	6	0	1	2	4	0	-	-	0	0	-	0	-
ILEUM (# examined)	10	10	10	10	10	9	10	10	5	0	3	5	5	0	5	0

	Main study (day 183)								Recovery (Day 196)							
	Males				Females				Males				Females			
MLN 9708 dose (mg/kg)	0	0.2	0.4	0.8	0	0.2	0.4	0.8/0.6	0	0.2	0.4	0.8	0	0.2	0.4	0.8/0.6
number of animals in study	10	10	10	10	10	10	10	10	5	5	5	5	5	5	5	5
Epithelial hyperplasia																
minimal	0	4	3	7	0	3	5	5	0	-	0	0	0	-	0	-
mild	0	0	3	1	0	1	4	1	0	-	0	0	0	-	0	-
moderate	0	0	0	0	0	0	1	2	0	-	0	0	0	-	0	-
TOTAL	**0**	**4**	**6**	**8**	**0**	**4**	**10**	**8**	**0**	**-**	**0**	**0**	**0**	**-**	**0**	**-**
Infiltrate, neutrophil																
minimal	0	4	3	9	0	1	9	3	0	-	0	0	0	-	0	-
mild	0	0	2	1	0	1	0	1	0	-	0	0	0	-	0	-
TOTAL	**0**	**4**	**5**	**10**	**0**	**2**	**9**	**4**	**0**	**-**	**0**	**0**	**0**	**-**	**0**	**-**
Single cell necrosis																
minimal	0	0	3	5	0	0	1	3	0	-	0	1	0	-	0	-
JEJUNUM (# examined)	10	10	10	10	10	10	10	10	5	0	0	5	5	0	5	0
Epithelial hyperplasia																
minimal	0	7	5	4	0	4	7	4	0	-	-	0	0	-	0	-
mild	0	0	4	6	0	1	2	5	0	-	-	0	0	-	0	-

	Main study (day 183)								Recovery (Day 196)							
	Males				Females				Males				Females			
MLN 9708 dose (mg/kg)	0	0.2	0.4	0.8	0	0.2	0.4	0.8/0.6	0	0.2	0.4	0.8	0	0.2	0.4	0.8/0.6
number of animals in study	10	10	10	10	10	10	10	10	5	5	5	5	5	5	5	5
TOTAL	**0**	**7**	**9**	**10**	**0**	**5**	**9**	**9**	**0**	**-**	**-**	**0**	**0**	**-**	**0**	**-**
Infiltrate, neutrophil																
minimal	0	2	7	4	0	0	4	5	0	-	-	0	0	-	0	-
mild	0	0	0	5	0	0	0	0	0	-	-	0	0	-	0	-
TOTAL	**0**	**2**	**7**	**9**	**0**	**0**	**4**	**5**	**0**	**-**	**-**	**0**	**0**	**-**	**0**	**-**
Single cell necrosis																
minimal	0	0	3	5	0	0	0	1	0	-	-	0	0	-	0	-
CECUM (# examined)	10	10	10	10	10	0	10	10	5	0	0	5	0	0	0	0
Epithelial hyperplasia																
minimal	0	0	1	5	0	-	0	2	1	-	-	0	-	-	-	-
mild	0	0	1	0	0	-	0	0	0	-	-	0	-	-	-	-
TOTAL	**0**	**0**	**2**	**5**	**0**	**-**	**0**	**2**	**1**	**-**	**-**	**0**	**-**	**-**	**-**	**-**
Infiltrate, neutrophil																
minimal	1	0	1	6	0	0	0	0	0	-	-	0	-	-	-	-
COLON (# examined)	10	0	10	10	10	10	10	10	5	0	0	5	5	0	5	0

	Main study (day 183)								Recovery (Day 196)							
	Males				Females				Males				Females			
MLN 9708 dose (mg/kg)	0	0.2	0.4	0.8	0	0.2	0.4	0.8/0.6	0	0.2	0.4	0.8	0	0.2	0.4	0.8/0.6
number of animals in study	10	10	10	10	10	10	10	10	5	5	5	5	5	5	5	5
Epithelial hyperplasia																
minimal	0	-	0	1	0	0	0	0	0	-	-	0	0	-	0	-
mild	0	-	0	2	0	0	0	0	0	-	-	0	0	-	0	-
TOTAL	**0**	-	**0**	**3**	**0**	**0**	**0**	**0**	**0**	-	-	**0**	**0**	-	**0**	-
Infiltrate, neutrophil																
minimal	0	-	0	2	0	2	1	2	0	-	-	0	0	-	0	-
mild	0	-	0	2	0	0	0	0	0	-	-	0	0	-	0	-
TOTAL	**0**	-	**0**	**4**	**0**	**2**	**1**	**2**	**0**	-	-	**0**	**0**	-	**0**	-
Single cell necrosis																
minimal	0	-	0	1	0	0	0	0	0	-	-	0	0	-	0	-
Perivasculitis, mononuclear																
minimal	0	-	0	1	0	0	0	0	0	-	-	0	0	-	0	-
RECTUM (# examined)	10	10	10	10	10	10	10	10	5	0	0	5	5	0	5	0
Infiltrate, neutrophil																
minimal	0	2	2	3	0	0	1	3	1	-	-	1	0	-	0	-

	Main study (day 183)								Recovery (Day 196)							
	Males				Females				Males				Females			
MLN 9708 dose (mg/kg)	0	0.2	0.4	0.8	0	0.2	0.4	0.8/0.6	0	0.2	0.4	0.8	0	0.2	0.4	0.8/0.6
number of animals in study	10	10	10	10	10	10	10	10	5	5	5	5	5	5	5	5
mild	0	0	0	3	0	0	0	0	0	-	-	0	0	-	0	-
TOTAL	**0**	**2**	**2**	**6**	**0**	**0**	**1**	**3**	**1**	-	-	**1**	**0**	-	**0**	-
Single cell necrosis																
minimal	0	0	1	5	0	0	0	0	0	-	-	0	0	-	0	-
GLANDULAR STOMACH (# examined)	10	10	10	10	10	0	10	10	5	0	0	5	0	0	0	0
Inflammation, subacute																
minimal	0	0	3	5	0	-	0	1	1	-	-	0	-	-	-	-
moderate	0	0	0	1	0	-	0	0	0	-	-	0	-	-	-	-
TOTAL	**0**	**0**	**3**	**6**	**0**	-	**0**	**1**	**1**	-	-	**0**	-	-	-	-
Erosion																
minimal	0	0	0	2	0	-	1	0	1	-	-	0	-	-	-	-
mild	0	0	0	1	0	-	0	0	0	-	-	0	-	-	-	-
TOTAL	**0**	**0**	**0**	**3**	**0**	-	**1**	**0**	**1**	-	-	**0**	-	-	-	-
SALIVARY GLAND, MANDIBULAR (#examined)	10	10	10	10	10	10	10	10	5	0	0	5	5	5	5	0

	Main study (day 183)								Recovery (Day 196)							
	Males				Females				Males				Females			
MLN 9708 dose (mg/kg)	0	0.2	0.4	0.8	0	0.2	0.4	0.8/0.6	0	0.2	0.4	0.8	0	0.2	0.4	0.8/0.6
number of animals in study	10	10	10	10	10	10	10	10	5	5	5	5	5	5	5	5
Increased mucin																
minimal	0	0	5	2	0	0	4	3	0	-	-	0	0	0	1	-
mild	0	0	1	3	0	0	0	1	0	-	-	0	0	0	0	-
moderate	0	0	0	3	0	0	0	0	0	-	-	0	0	0	0	-
TOTAL	**0**	**0**	**6**	**8**	**0**	**0**	**4**	**4**	**0**	-	-	**0**	**0**	**0**	**1**	-
LYMPH NODE, MESENTERIC (# examined)	10	10	10	10	10	10	10	10	5	0	3	5	5	0	5	0
Necrosis, lymphoid																
minimal	0	1	3	6	0	1	3	6	0	-	0	0	0	-	0	-
mild	0	0	0	0	0	0	0	1	0	-	0	0	0	-	0	-
TOTAL	**0**	**1**	**3**	**6**	**0**	**1**	**3**	**7**	**0**	-	**0**	**0**	**0**	-	**0**	-
Depletion, lymphoid																
minimal	1	2	2	6	0	0	0	0	0	-	0	2	0	-	0	-
mild	0	0	3	1	0	0	0	0	0	-	0	1	0	-	0	-
TOTAL	**1**	**2**	**5**	**7**	**0**	**0**	**0**	**0**	**0**	-	**0**	**3**	**0**	-	**0**	-
Infiltrate, neutrophil																
minimal	0	0	0	3	0	0	0	0	0	-	0	0	0	-	0	-
SPLEEN (# examined)	10	10	10	10	10	0	10	10	5	1	3	5	0	0	0	0
Infiltrate, neutrophil																

	Main study (day 183)								Recovery (Day 196)							
	Males				Females				Males				Females			
MLN 9708 dose (mg/kg)	0	0.2	0.4	0.8	0	0.2	0.4	0.8/0.6	0	0.2	0.4	0.8	0	0.2	0.4	0.8/0.6
number of animals in study	10	10	10	10	10	10	10	10	5	5	5	5	5	5	5	5
minimal	4	1	5	3	2	-	3	4	2	0	0	1	-	-	-	-
mild	0	0	2	7	0	-	0	1	0	0	0	0	-	-	-	-
TOTAL	**4**	**1**	**7**	**10**	**2**	-	**3**	**5**	**2**	**0**	**0**	**1**	-	-	-	-
SPINAL CORD (# examined)	10	0	0	10	10	0	0	10	0	0	0	0	0	0	0	0
Axonal degeneration																
minimal	6	-	-	9	6	-	-	6	-	-	-	-	-	-	-	-
Perivasculitis, mononuclear, meninges																
minimal	0	-	-	1	0	-	-	0	-	-	-	-	-	-	-	-
TESTES (# examined)	10	0	10	10	0	0	0	0	5	0	0	5	0	0	0	0
Spermatids, retention																
minimal	0	-	0	1	-	-	-	-	0	-	-	0	-	-	-	-

Toxicokinetics

Blood samples (~0.5 ml) for toxicokinetic evaluation were taken from 3 animals per sex per group at each time point. Blood was processed to plasma. 250 µl of each plasma sample was mixed with 15 µl of 30% ascorbic acid and stored frozen at -65 to -85°C. Frozen samples were shipped to [redacted] (b) (4) for analysis. MLN9708 is a prodrug for the active MLN2238. Toxicokinetic values reflect measurement of MLN2238. MLN238 concentration was determined using a validated HPLC/MS/MS method and toxicokinetic analysis was conducted using Phoenix™ WinNonlin®, Version 6.2.

MLN9708 was rapidly absorbed, with Tmax values generally between 0.5 – 1 h post-dose at all time points. MLN9708 exposure increased approximately proportionally with dose in males and females after both the first dose of cycle 1 and cycle 7 (Figure 10, Table 24). Evidence for modest accumulation was observed in LD males and females only (accumulation factor: 1.3 – 1.9) at Day 168. Mean Cmax values were ~3 –fold higher in HD females vs. HD males after the initial MLN9708 dose. AUC0-168 values were 1.4-fold higher in females at this time point. Mean Cmax values were also increased in females vs. males at the Day 14 and Day 42 time points (data not shown). The Applicant's notion that the 4 premature deaths observed in HD females are related to elevated Cmax appears to be reasonable. No clear gender differences were observed in Cmax or AUC0-168 at Day 168.

Figure 10.Plasma MLN2238 concentration vs. time curves after MLN9708 administration to SD rats

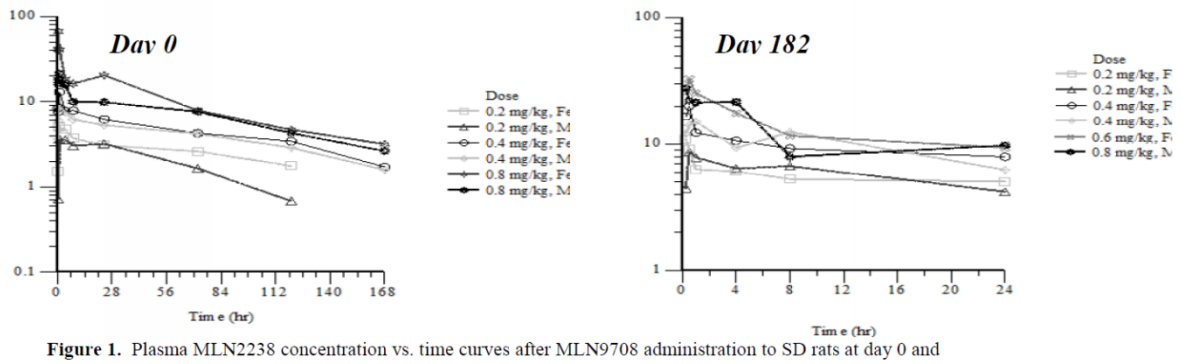


Figure 1. Plasma MLN2238 concentration vs. time curves after MLN9708 administration to SD rats at day 0 and day 182.

(Excerpted from the submission)

Table 24. Summary toxicokinetics for 6 month repeat dose toxicology study in rat

Dose (mg/kg)	C_{max} (ng/ml)						AUC_{0-168} (ng*hr/ml)					
	0.2 mg/kg		0.4 mg/kg		0.8 mg/kg		0.2 mg/kg		0.4 mg/kg		0.8/0.6 mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Day 0	3.68	5.12	8.97	13.0	21.4	66.4	264	374	655	742	1150	1660
Day 168	5.42	6.55	19.2	21.3	62.1	44.7	489	476	754	803	1200	1070

(Excerpted from the submission)

Dosing Solution Analysis

The mean concentrations of MLN9708 in solution analyzed during the study were within acceptability criteria of ±10% of nominal concentrations, indicating accurate formulation. No test article was detected in the vehicle administered to the control group. Stability was not tested in this study, but a previous study was cited stating that test article

stability following 24 hours of room temperature storage or 14 days of refrigerated storage was assessed and verified in formulations ranging in concentration from 0.005 to 0.2 mg MLN9708/mL (Study No. (b) (4)-416108).

Table 25. Dosing solution analysis (concentration) for 6 month repeat dose toxicology study in rats

	Mean Concentration, mg/mL (% of Target)			
Date of Preparation	0.04 mg/mL	0.08 mg/mL	0.12 mg/mL	0.16 mg/mL
04-SEP-2013	0.0397 (99.2)	0.0802 (100)	NA	0.159 (99.3)
18-SEP-2013	0.0402 (100)	0.0806 (101)	NA	0.158 (98.7)
30-OCT-2013	0.0407 (102)	0.0809 (101)	0.121 (101)	0.162 (101)
19-FEB-2014	0.0402 (100)	0.0796 (99.5)	0.120 (99.8)	0.155 (97.0)
05-MAR-2014	0.0414 (103)	0.0819 (102)	0.121 (101)	0.165 (103)

NA = Not applicable

(Excerpted from the submission)

Study title: A 9-month oral gavage toxicity study of MLN9708 with a 2-week recovery period in Beagle dogs

The following study was initially reviewed under IND (b) (4) by Matt Whittaker PhD and parts of it were reformatted to fit this NDA review

Study no.:	(b) (4) 416164
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 29, 2013
GLP compliance:	Signed and Included
QA statement:	Signed and Included
Drug, lot #, and % purity:	MLN9708; Lot #101296; 69.3% purity

Key Study Findings

- There were no mortalities during the study.
- Toxicologically significant ixazomib hematology findings included higher mean values of absolute counts for total white blood cells, neutrophils, and monocytes and lower mean counts for lymphocytes at 0.2 mg/kg dose group.
- Toxicologically significant ixazomib related changes in clinical chemistry parameters included elevated AST levels and lower phosphorous levels at the 0.20 mg/kg/dose group.
- Histopathology findings included neutrophil infiltration in the gastrointestinal tract (stomach, intestines, peyers patch) and mesenteric lymph nodes and erosion in the stomach at 0.2 mg/kg dose group. Findings in the nervous system primarily at 0.2 mg/kg dose group included neuronal degeneration of the sympathetic (celiac and stellate), dorsal root, and end organ (salivary gland) ganglia, and minimal secondary axonal/nerve fiber degeneration of the peripheral nerves (vagus and sciatic nerves, dorsal roots and mixed spinal nerves) and ascending tracts in the dorsal columns of the spinal cord, and in white matter tracts in the medulla oblongata of the brain, gliosis of the dorsal column of the spinal cord and the white matter tracts in the brain.
- Histopathology findings still present at recovery were limited to the nervous system and included nerve fiber degeneration of the dorsal root ganglia and an increase in axonal degeneration in the dorsal columns of the spinal cord.

Reference ID: 3844453

Methods

Doses:	0.05, 0.1, 0.2 mg/kg
Frequency of dosing:	Once weekly for 3 weeks with each cycle separated by a 13-day nondosing period. 10 x 28 day cycles.
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	55 mM citrate buffer and 0.45% NaCl, pH 5.8
Species/Strain:	Canis lupus familiaris, Beagle dogs
Number/Sex/Group:	3/sex/group, 3/sex per recovery excluding 0.05 dose group
Age:	12-14 months
Weight:	9.1 kg to 12.5 kg (males) or 7.1 kg to 8.9 kg (females)
Satellite groups:	N/A
Unique study design:	N/A
Deviation from study protocol:	None impacted study results or objectives

Table 26. Study design for 9 month repeat dose toxicology study in dogs

MLN9708 Dose (mg/kg)	Total animals (dogs/sex)	Main study (dogs/sex)	Recovery [+ 2 wks] (dogs/sex)
0	6	3	3
0.05	3	3	0
0.10	6	3	3
0.20	6	3	3

(Excerpted from the submission)

Observations and Results

Mortality

There were no mortalities during the study.

Clinical Signs

Unremarkable.

Body Weights

Unremarkable

Feed Consumption

Unremarkable.

Ophthalmoscopy

Visual examinations were not performed.

Reference ID: 3844453

ECG

Unremarkable

Hematology

Table 27. Hematology findings for 9 month repeat dose toxicity study in dogs (percent change from control)

Parameter	Interval (Week)	Males (mg/kg/dose) 0.05	0.10	0.20	Females (mg/kg/dose) 0.05	0.10	0.20
WBC	38	n	n	40	n	n	37*
	40	n	n	n	n	n	n
Abs Neutrophils	38	n	n	62	n	n	65**
	40	n	n	n	n	n	n
Abs Monocytes	38	n	n	87	n	n	127**
	40	n	n	n	n	n	n
Abs. Lymphocytes	38	n	n	n	n	n	-26
	40	n	n	n	n	n	n

Abs = absolute; n = no noteworthy findings – or – no test article-related effect.
* Significantly different from the control group at 0.05 using Dunnett's test
** Significantly different from the control group at 0.01 using Dunnett's test

(Excerpted from the submission)

There were no significant changes observed at recovery (Week 40) compared to the main study (Week 38).

Clinical Chemistry

Table 28. Clinical chemistry findings for 9 month repeat dose toxicity study in dogs (percent change from control)

Parameter	Interval (Week)	Males (mg/kg/dose) 0.05	0.10	0.20	Females (mg/kg/dose) 0.05	0.10	0.20
Phosphorus	38	n	n	-19	n	n	-20*
	40	n	n	n	n	n	n
AST	38	n	n	36**	n	n	n
	40	n	n	n	n	n	n

AST = aspartate aminotransferase; n = no noteworthy findings or no test article-related effect.
*Significantly different from the control group at 0.05 using Dunnett's test
**Significantly different from the control group at 0.01 using Dunnett's test

(Excerpted from the submission)

There were no significant changes observed at recovery (Week 40) compared to the main study (Week 38).

Urinalysis

Urinalysis was not conducted

Gross Pathology

Dark red areas in the stomach of animal 4156 (0.20 mg/kg/dose group female). In animal 4156 dark red areas correlated microscopically with neutrophilic infiltrates in the stomach. Three high dose male animals (0.2 mg/kg) had reported dark red discoloration of the colon without microscopic correlates.

Organ Weights

Unremarkable

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

Table 29. Histological findings for 9 month repeat dose toxicity study in dogs

Histopathology findings in lymphoid and GI after 10 cycles

	Main study (Week 38)								Recovery (Week 40)							
	Males				Females				Males				Females			
MLN 9708 dose (mg/kg)	0	0.05	0.10	0.20	0	0.05	0.10	0.20	0	0.05	0.10	0.20	0	0.05	0.10	0.20
number of animals in study	3	3	3	3	3	3	3	3	3	0	3	3	3	0	3	3
GALT [PEYER'S PATCH] (# examined)	3	3	3	3	3	3	3	3	3	0	3	3	3	0	3	3
Infiltrate, neutrophil																
mild	0	0	0	0	0	0	0	1	0	-	0	0	0	-	0	0
Lymphoid depletion																
mild	0	0	0	0	0	0	0	1	0	-	0	0	0	-	0	0
LYMPH NODE, MESENTERIC (# examined)	3	3	3	3	3	3	3	3	3	0	3	3	3	0	3	3
Infiltrate, neutrophil																
minimal	0	0	0	1	0	0	0	0	0	-	0	0	0	-	0	0
mild	0	0	0	0	0	0	0	1	0	-	0	0	0	-	0	0
Total	0	0	0	1	0	0	0	1	0	-	0	0	0	-	0	0
Lymphoid depletion																

	Main study (Week 38)								Recovery (Week 40)							
	Males				Females				Males				Females			
MLN 9708 dose (mg/kg)	0	0.05	0.10	0.20	0	0.05	0.10	0.20	0	0.05	0.10	0.20	0	0.05	0.10	0.20
number of animals in study	3	3	3	3	3	3	3	3	3	0	3	3	3	0	3	3
minimal	0	0	0	1	0	0	0	0	0	-	0	0	0	-	0	0
INTESTINE [DUODENUM, ILEUM, RECTUM] (# examined)	3	3	3	3	3	3	3	3	3	0	3	3	3	0	3	3
Infiltrate, neutrophil																
minimal	0	2	0	1	0	0	1	2	**0**	-	**0**	**0**	**0**	-	**1**	**1**
STOMACH (# examined)	3	3	3	3	3	3	3	3	3	0	3	3	3	0	3	3
Erosion																
minimal	0	0	0	1	0	0	0	0	0	-	0	0	0	-	0	0
Infiltrate, neutrophil																
minimal	0	0	1	1	0	0	1	2	0	-	0	0	0	-	0	0

Table 30. Histological findings for 9 month repeat dose toxicity study in dogs (nervous system-Week 38)

	Males				Females			
Dosage (mg/kg/dose):	**0**	**0.05**	**0.10**	**0.20**	**0**	**0.05**	**0.10**	**0.20**
Dorsal root ganglion, lumbar [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Degeneration, neuronal	0	0	0	3	0	0	0	2
Minimal	-	-	-	3	-	-	-	2
Degeneration, nerve fiber	0	0	0	1	0	1	0	2
Minimal	-	-	-	1	-	1	-	2
Stellate ganglion [a]	**3**	**3**	**3**	**2**	**3**	**3**	**3**	**3**
Degeneration, neuronal	0	0	0	1	0	0	1	3
Minimal	-	-	-	1	-	-	1	3
Degeneration, nerve fiber	0	0	0	2	0	0	0	0
Minimal	-	-	-	2	-	-	-	-
Celiac ganglion [a]	**3**	**2**	**3**	**3**	**3**	**3**	**3**	**3**
Degeneration, neuronal	0	0	0	3	0	0	0	3
Minimal	-	-	-	3	-	-	-	3
Peripheral autonomic ganglion (salivary gland) [a,b]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Degeneration, neuronal	0	0	0	3	0	0	0	2
Minimal	-	-	-	3	-	-	-	2
Sciatic nerve [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Degeneration, nerve fiber	0	1	0	3	0	0	0	3
Minimal	-	1	-	3	-	-	-	3
Vagus nerve [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Degeneration, nerve fiber	0	0	0	1	0	0	0	1
Minimal	-	-	-	1	-	-	-	1
Brain[a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Degeneration, white matter tract, medulla	0	0	0	0	0	0	0	1
Minimal	-	-	-	-	-	-	-	1
Gliosis	0	0	0	0	0	0	0	1
Minimal	-	-	-	-	-	-	-	1
Spinal cord [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Degeneration, axonal, dorsal columns	0	0	0	3	0	1	0	3
Minimal	-	-	-	3	-	1	-	3
Gliosis, dorsal column	0	0	0	1	0	0	0	1
Minimal	-	-	-	1	-	-	-	1

[a] = Number of animals examined from each group.
[b] = Number of animals with lesion in the salivary gland

(Excerpted from the submission)

Table 31.Histological findings for 9 month repeat dose toxicity study in dogs (nervous system-recovery Week 40)

	Males				Females			
Dosage (mg/kg/dose):	**0**	**0.05**	**0.10**	**0.20**	**0**	**0.05**	**0.10**	**0.20**
Dorsal root ganglion, lumbar [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Degeneration, nerve fiber	0	0	0	3	0	0	0	1
Minimal	-	-	-	3	-	-	-	1
Spinal cord [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Degeneration, axonal, dorsal columns	0	0	0	1	1	0	0	2
Minimal	-	-	-	1	1	-	-	2

[a] = Number of animals examined from each group.
[b] = Number of animals with lesion in the salivary gland

(Excerpted from the submission)

Special Evaluation

Functional observation battery- nervous system

Table 32. Time points of conduct of FOB assessments in 10 cycle study with MLN9708 in beagle dogs.

Time point	Description
Week 8	Prior to **Cycle 3**
Week 10	~24 – 36 hrs after 3d dose **Cycle 3**
Week 21	Between 2nd and 3d doses of **Cycle 6**
Week 37	Between 2nd and 3d doses of **Cycle 10**
Week 39	Prior to **recovery necropsy**

(Excerpted from the submission)

Motor, sensory, and autonomic pathways were examined to assess neurological function. The parameters evaluated are summarized below. Each parameter was evaluated according to an established scoring system. The conduct of the FOB was consistent with the proposal submitted by the Applicant on 2/11/14.

Table 33. Parameters evaluated in FOB assessments in beagle dogs.

Home cage observations	
General posture	Salivation
Behavior	Lacrimation
General demeanor	Palpebral fissure
Head posture	body weight
Tremors/convulsions	Excreta
Emesis	

(Excerpted from the submission)

Functional Observations and Measurements	
Respiration rate	Righting reflex
Respiration pattern	Triceps reflex
Perineal reflex	Patellar reflex
Temperature	Sensitivity response
Auditory response	Cliff avoidance
Pinna reflex	Proprioceptive positioning
Menace reaction	Posterior extensor thrust
Pupillary size	Wheel barrowing
Pupillary reflex	Hemistanding/hemiwalking
Pathologic nystagamus	Capillary refill time
Palepbral reflex	Jaw and tongue examination
Ocular position	Gag reflex

(Excerpted from the submission)

There was no evidence for treatment related effects on any parameter. A diminished or absent perineal reflex was observed in a small number (1-2) of both control and high dose male and female dogs animals throughout the study period. All other open field and functional observations were scored as present or normal for males and females of all dose groups.

There was no evidence for MLN9708-evoked functional deficits in any dogs in the study.

Toxicokinetics

On Days 0, 14 and 252, whole blood samples from all animals were collected predose and at approximately 0.25, 0.5, 1, 4, 8, 24, 72, 120, and 168 hours postdose. Samples were also collected at the conclusion of the recovery period on Day 266 at approximately 0.25, 0.5, 1, 4, 8, and 24 hours postdose. MLN9708 is a prodrug for the active MLN2238. Toxicokinetic values reflect measurement of MLN2238. Plasma MLN2238 concentrations were determined using a validated HPLC/MS/MS method. The lower limit of quantitation was 2.0 ng/ml. Toxicokinetic analysis was conducted using Phoenix™ WinNonlin®, Version 6.2. There were no differences between males and females with respect to Cmax or AUC0-168.
Mean plasma Tmax values were less than 1 hour throughout the duration of the study. Mean plasma Cmax and AUC0-168 increased in an approximately dose-proportional manner at all time points (Table 34). There was no evidence for significant accumulation over the course of the 9 month study at any dose level.

Table 34. Summary toxicokinetics for 9 month repeat dose toxicology study in dogs

Dose (mg/kg)	C_{max} (ng/ml)						AUC_{0-168} (ng*hr/ml)					
	0.05 mg/kg		0.10 mg/kg		0.20 mg/kg		0.05 mg/kg		0.10 mg/kg		0.20 mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Day 0	29.8	48.0	107	106	249	305	613	800	2070	1730	3370	3230
Day 252	62.2	52.1	134	137	334	332	953	914	2170	1710	3630	4170

(Excerpted from the submission)

Safety concerns stem from the steep dose-toxicity relationship with MLN9708 in the chronic rat and dog toxicology studies. There was a less than 1.5-fold separation in systemic exposure between the limit dose and a dose associated with mortality due to drug-induced injury of the liver and GI tract in the rat (Table 35). In dogs, there was an approximately 2-fold separation in exposure between the NOAEL and doses associated with extensive neuronal pathology in the CNS and PNS.

Table 35. Evidence for steep dose-toxicity relationship with MLN9708 in chronic rat and dog toxicology

Study	Species	Route	NOAEL*		Toxic dose	
			Dose (mg/kg)	Mean AUC_{0-168} (µg*h/ml)	Dose (mg/kg)	Mean AUC_{0-168} (µg*h/ml)
(b) (4) 416165	Rat	Oral	0.4	0.78	0.8[a]	1.14
416164	Dog	Oral	0.1	1.9	0.2[b]	3.9

*No NOAEL was identified in rat study. Value listed is dose used to establish limits to clinical dosing

[a] Mortality due to liver, GI pathology

[b] Neuronal degeneration in spinal cord & peripheral nerves

(Excerpted from the submission)

Dosing Solution Analysis

The mean concentrations of MLN9708 in solution analyzed during the study were within acceptability criteria of ±10% of nominal concentrations, indicating accurate formulation. No test article was detected in the vehicle administered to the control group. Stability was not tested in this study, but a previous study was cited stating that test article stability following 24 hours of room temperature storage or 14 days of refrigerated storage was assessed and verified in formulations ranging in concentration from 0.005 to 0.2 mg MLN9708/mL (Study No. (b) (4) 416108).

Table 36. Dosing solution analysis (concentration) for 9 month repeat dose toxicology study in dogs

	Mean Concentration, mg/mL (% of Target)		
Date of Preparation	0.01 mg/mL	0.02 mg/mL	0.04 mg/mL
28-Aug-2013	0.0102 (102)	0.0203 (102)	0.0405 (101)
11-Sep-2013	0.0101 (101)	0.0203 (102)	0.0407 (102)
07-May-2014	0.00988 (98.8)	0.0198 (98.8)	0.0396 (99.1)
07-May-2014 [a]	0.00985 (98.5)	0.0197 (98.5)	0.0397 (99.2)
08-May-2014	0.00982 (98.2)	0.0197 (98.7)	0.0396 (99.1)
21-May-2014	0.0113 (113)	0.0229 (114)	0.0386 (96.4)
21-May-2014 [a]	0.0111 (111)	0.0219 (109)	0.0384 (95.9)
22-May-2014	0.0989 (98.9)	0.0198 (99.1)	NA

NA = Not applicable

[a] Backup samples.

(Excerpted from the submission)

7 Genetic Toxicology

7.1 In vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Salmonella E.coli/Mammalian Microsome Reverse Mutation Assay

Study no.:	(b) (4) 08-152
Study report location:	4.2.3.3.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 2, 2008
GLP compliance:	Signed and Included
QA statement:	Signed and Included
Drug, lot #, and % purity:	MLN-2238, Lot# 001-D, purity 98.24%

Key Study Findings

- MLN-2238 was negative in the in tester strains of Salmonella or E. coli in the presence and absence of S-9 mix up to 5000 µg/plate, under the conditions tested.

Methods

Strains:	*Salmonella typhimurium* :TA98, TA100, TA1535 and TA1537 *Escherichia coli:* WP2 uvrA
Concentrations in definitive study:	100, 250, 500, 1000, 2500, and 5000 µg/plate with TA98; 50, 100, 250, 500, 1000 and 2500 µg/plate WP2 uvrA; 25, 50, 100, 250, 500 and 1000 with TA1537 and TA1535, and at 10, 25, 50, 100, 250 and 500 µg/plate with TA100.
Basis of concentration selection:	Cytotoxicity observed in the initial assay or up to the limit dose (5000 µg/plate) recommended by regulatory guidelines.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	With S9: 2-Aminoanthracene, Without S9: Sodium Azide, ICR-191 acridine, 2-Nitrofluorene, 4-Nitroquinoline-N-oxide
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Plate incorporation: 48 hours at 37±1°C

Analysis

After incubation, colonies were counted electronically for bacterial colony formation using a ProtoCol automatic colony counter, Model number 60000, software version 2.05. Cytotoxicity was assessed by examining bacterial lawn density and number of spontaneous revertants per plate.

Reference ID: 3844453

Criteria for Positive Response:

Results were considered positive if the data for any treatment level showed a dose dependent a response with at least 2 times the concurrent vehicle control for TA100 and 3 times the concurrent vehicle control for TA1535, TA1537, TA98 and WP2 *uvrA*.

Study Validity

- The vehicle and the positive control values (± S9 mix) for each tester strain were within the laboratory historical ranges.
- Vehicle control plates displayed normal growth (i.e., normal background lawn) in the ± S9 mix.
- Selection of the tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICHS2A, April 1996).
- The highest concentration tested was 5000 µg/plate, which allowed maximum exposure.
- The appropriate positive control compounds (± S9 mix) produced clear, unequivocal increases in the number of revertant colonies.
- Tester strains demonstrated their appropriate genetic markers for strain integrity.

Results

- MLN 9708 was cytotoxic (i.e., decrease in spontaneous revertants and/or background lawn) to all strains tested at different concentrations. MLN 9708 concentrations ≤ 2500 µg/plate showed no signs of bacterial mutagenicity. As shown in the table below, MLN 9708 was not mutagenic in tester strains of Salmonella or E. coli in the presence or absence of S-9 mix, under the conditions tested.

Reference ID: 3844453

Table 37. Summary results of initial assay for MLN2238 in the Salmonella/E.Coli mammalian microsome reverse mutation assay

		REVERTANT COLONIES PER PLATE—Mean (SEM)									
Treatment	µg/plate	TA1537		TA98		TA100		TA1535		WP2*uvrA*	
WITHOUT ACTIVATION											
DMSO	50 µL	8	(1)	10	(0)	96	(3)	14	(1)	32	(4)
ICR	0.5	354	(42)								
2NF	2.5			842	(59)						
SA	1.0					548	(43)	384	(5)		
NQNO	2.0									944	(21)
MLN2238	25	8	(3)	13	(2)	80	(2)	11	(2)	32	(3)
	50	8	(2)	15	(2)	73	(4)	8	(0)	30	(3)
	100	9	(2)	11	(1)	60	(2)	12	(3)	17	(2)
	250	5	(1)	11	(3)	41	(5)	7	(1)	23	(2)
	500	2	(1)	7	(1)	27	(5)	7	(0)	22	(2)
	1000	1	(1)	6	(2)	17	(2)	6	(1)	16	(2)
	2500[b]	0	(0)	2	(2)	0	(0)	0	(0)	10	(3)
	5000[a]	0	(0)	0	(0)	0	(0)	0	(0)	8	(3)
WITH ACTIVATION											
DMSO	50 µL	8	(1)	14	(3)	102	(3)	12	(1)	39	(1)
2AA	2.5	94	(3)	2143	(22)	942	(20)	193	(12)		
2AA	10.0									562	(33)
MLN2238	25	7	(0)	15	(3)	72	(2)	8	(0)	35	(2)
	50	5	(1)	19	(2)	74	(7)	9	(2)	25	(1)
	100	6	(2)	18	(2)	48	(1)	6	(1)	28	(4)
	250	5	(1)	15	(1)	23	(4)	8	(3)	19	(4)
	500	4	(2)	14	(2)	18	(1)	6	(1)	14	(1)
	1000	3	(1)	10	(1)	18	(1)	3	(2)	12	(2)
	2500[c]	1	(0)	4	(1)	21	(6)	0	(0)	14	(2)
	5000[a]	0	(0)	0	(0)	0	(0)	0	(0)	11	(2)

DMSO = Dimethylsulfoxide; 2AA = 2-Aminoanthracene; 2NF = 2-Nitrofluorene;
ICR = ICR-191 Acridine; NQNO = 4-nitroquinoline-N-oxide; SA = Sodium azide;
SEM= standard error of the mean

a: Reduced background in strains TA1537, TA98, TA100 and TA1535, plates not counted.

b: Reduced background in strains TA1537, TA100 and TA1535, plates not counted.

c: Reduced background in strainTA1535, plates not counted.

(Excerpted from the submission)

Table 38. Summary results of confirmatory assay for MLN2238 in the Salmonella/E. coli mammalian microsome reverse mutation assay

		REVERTANT COLONIES PER PLATE—Mean (SEM)									
Treatment	µg/plate	TA1537		TA98		TA100		TA1535		WP2*uvrA*	
WITHOUT ACTIVATION											
DMSO	50 µL	6	(3)	12	(2)	102	(11)	15	(2)	29	(2)
ICR	0.5	311	(14)								
2NF	2.5			749	(49)						
SA	1.0					572	(34)	476	(29)		
NQNO	2.0									757	(41)
	10					86	(2)				
	25	5	(1)			84	(4)	13	(1)		
	50	5	(0)			74	(11)	16	(5)	21	(3)
	100	4	(1)	12	(3)	60	(1)	17	(4)	26	(4)
MLN2238	250	3	(2)	14	(3)	48	(11)	11	(2)	17	(1)
	500	1	(1)	12	(0)	28	(2)	10	(2)	16	(2)
	1000	1	(1)	2	(1)			5	(1)	14	(2)
	2500			3	(1)					7	(1)
	5000[a]			0	(0)						
WITH ACTIVATION											
DMSO	50 µL	7	(1)	17	(2)	102	(5)	11	(3)	39	(4)
2AA	2.5	298	(17)	2005	(106)	1615	(156)	204	(12)		
2AA	10.0									509	(66)
	10					89	(8)				
	25	5	(1)			70	(9)	8	(2)		
	50	6	(1)			65	(8)	8	(1)	28	(1)
	100	8	(2)	12	(3)	46	(1)	7	(1)	23	(3)
MLN2238	250	5	(1)	12	(1)	27	(6)	5	(1)	18	(3)
	500	2	(1)	6	(1)	30	(8)	4	(2)	14	(5)
	1000	0	(0)	9	(2)			3	(1)	16	(3)
	2500			1	(0)					9	(3)
	5000[a]			0	(0)						

DMSO = Dimethylsulfoxide; 2AA = 2-Aminoanthracene; 2NF = 2-Nitrofluorene;
ICR = ICR-191 Acridine; NQNO = 4-nitroquinoline-N-oxide; SA = Sodium azide;
SEM= standard error of the mean
a: Reduced background in strain TA98, plates not counted.

(Excerpted from the submission)

7.2 In Vitro Assays in Mammalian Cells

Study title: In vitro Chromosome Aberration Test in Cultured Human Peripheral Blood Lymphocytes

Study no.:	(b) (4) 12-367
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 12, 2013
GLP compliance:	Signed and Included
QA statement:	Signed and Included
Drug, lot #, and % purity:	MLN-9708; lot # 101296; purity 99.1%

Key Study Findings

- MLN9708 was positive for inducing structural aberrations in HPBL with and without metabolic activation, under the conditions tested.

Methods

Cell line:	Human peripheral blood lymphocytes (HPBL)
Concentrations in definitive study:	Non-activated treatment (-S9): 3 hour s: 0.010, 0.030, 0.050, 0.10, 0.25, 0.50, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10, 12, 15, and 20 µg/mL 22 hours: 0.010, 0.020, 0.030, 0.040, 0.050, 0.055, 0.060, 0.065, 0.070, 0.075,0.080, 0.085, 0.090, 0.095, 0.10, 0.20, 0.30, 0.40, and 0.50 µg/mL S9-activated treatment (+S9): 3 hours: 0.10, 0.30, 0.50, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10, 12, 15, 20, 25, 30, 35, 40, 45, and 50 µg/mL
Basis of concentration selection:	Cytotoxicity tests were performed to select doses for the definitive assay. The concentrations tested ranging from 0.00305 to 50 µg/mL. Cytotoxicity was observed at MLN-9708 at concentrations of 0.0977 (3 hour and 22 hour) and 6.25 µg/mL with or without S9 mix, respectively;
Negative control:	Sterile distilled water
Positive control:	Mitomycin C for –S9 3 hour and 22 hour treatment Cyclophosphamide for +S9 3 hour treatment
Formulation/Vehicle:	Sterile distilled water
Incubation & sampling time:	3 hour treatment with 19 hour recovery for -S9/+S9

Reference ID: 3844453

22 hour treatment with no recovery for –S9

Table 39. Concentrations of MLN-9708 evaluated for chromosomal aberrations assay in HPBL

Treatment	Treatment Conditions and Concentrations		
	3 h without S9	3 h with S9	22 h without S9
Sterile Water (%)	1.0	1.0	1.0
Mitomycin C (µg/mL)	0.80	--	0.30
Cyclophosphamide (µg/mL)	--	20	--
MLN9708 (µg/mL)	0.010 0.050 0.10	35 40 45	0.050 0.065 0.075 0.080

(Excerpted from the submission)

Analysis

Approximately 2 hours prior to harvest, Colcemid® (0.1 µg/mL, final concentration in culture) was added to each culture to arrest cells in metaphase. Cells were treated with hypotonic solutions and fixed with 3:1 methanol: glacial acetic acid. Slides were prepared, and stained analysis.

Cytotoxicity

The number of mitotic cells in at least 500 cells from each culture was enumerated.

Chromosome aberrations

Coded slides were analyzed for structural chromosomal aberrations from a total of 200 metaphase cells and from a total of 400 cells for numerical aberrations from each concentration.

Study Validity

The study was considered valid based on meeting the following criteria for a valid test set in the protocol.

- The frequency of cells with structural chromosome aberrations in the vehicle control groups were within the historical range for vehicle controls.
- The percentage of cells with chromosome aberrations in each positive control group were statistically increased ($p \leq 0.05$, Fisher's Exact test), relative to the appropriate vehicle controls.

Criteria for Positive Response:

The test article would be considered positive for inducing chromosomal aberrations if a significant increase ($p \leq 0.01$) in the number of cells with chromosomal aberrations is observed at one or more dose levels, or if a significant increase ($p \leq 0.05$) in the number of cells with chromosomal aberrations is observed at two or more dose levels.

Results

- Substantial toxicity (≥ 50% reduction in mitotic index relative to the vehicle control) was observed in all 3 treatment conditions.
- MLN-9708 induced statistically significant increase in the number of structural aberrations with dose response at more than one concentration in the +S9 3 and 22 hour treatments and at one concentration in the –S9 3 hour treatment.
- MLN-9708 was negative for increases in cells with numerical aberration (polypoloidy and endoreduplications).

Table 40. Cytotoxicity and aberration summary: 3 hour incubation without metabolic activation

Treatment	% Mitotic Reduction	% Cells w/Abs	% Cells w/>1 Abs	% Endo Cells	% Polyploid Cells
Sterile Water (1%)	0	1.5	0.0	0.0	0.0
MMC 0.8 µg/mL	51	73.2 *	46.3 *	0.0	0.3
MLN9708					
0.010 µg/mL	3	0.0	0.0	0.0	0.0
0.050 µg/mL	9	1.5	0.5	0.0	0.0
0.10 µg/mL	57	11.5 *	1.5	0.0	0.5

Endo = Endoreduplicated cells

Abs = Aberrations MMC = Mitomycin C

Percent Aberrant cells: * $p \leq 0.01$ using Fisher's Exact Test

(Excerpted from the submission)

Table 41. Cytotoxicity and aberration summary: 22 hour incubation without metabolic activation

Treatment	% Mitotic Reduction	% Cells w/Abs	% Cells w/>1 Abs	% Endo Cells	% Polyploid Cells
Sterile Water (1%)	0	0.5	0.0	0.0	0.0
MMC 0.3 µg/mL	60	65.2 *	32.6 *	0.0	0.0
MLN9708					
0.050 µg/mL	9	100.0 *	100.0 *	_ [a]	_ [a]
0.065 µg/mL	34	0.0	0.0	0.0	0.3
0.075 µg/mL	45	6.0 *	1.0	0.0	0.8
0.080 µg/mL	58	9.5 *	3.0 **	0.0	0.5

Endo = Endoreduplicated cells

Abs = Aberrations MMC = Mitomycin C

Percent Aberrant cells * $p \leq 0.01$, ** $p \leq 0.05$ using Fisher's Exact Test

[a] Chromosomes pulverized, unable to score for polyploid and endoreduplication

(Excerpted from the submission)

Table 42. Cytotoxicity and aberration summary: 3 hour incubation with metabolic activation

Treatment	% Mitotic Reduction	% Cells w/Abs	% Cells w/>1 Abs	% Endo Cells	% Polyploid Cells
Sterile Water (1%)	0	0.5	0.0	0.0	0.5
CP 20 µg/mL	18	23.6 *	2.4	0.0	0.8
MLN9708					
35 µg/mL	-3	5.5 *	1.0	0.0	0.5
40 µg/mL	-15	5.0 **	1.0	0.0	0.3
45 µg/mL	56	18.5 *	6.8 *	0.0	0.5

Endo = Endoreduplicated cells
Abs = Aberrations CP = Cyclophosphamide
Percent Aberrant cells: * $p \leq 0.01$; ** $p \leq 0.05$ using Fisher's Exact Test
(Excerpted from the submission)

7.3 In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: MLN9708: A Single-Dose Oral (Gavage) Bone Marrow Micronucleus Evaluation in CD-1 Mice

Study no: (b) (4)-416152
Study report location: 4.2.3.3.2
Conducting laboratory and location: (b) (4)
Date of study initiation: October 25, 2012
GLP compliance: Signed and Included
QA statement: Signed and Included
Drug, lot #, and % purity: MLN-9708; Lot # 21E004; purity 99.0%

Key Study Findings

• MLN-9708 was negative in the micronucleus test in the bone marrow cells of male mice.

Methods

Doses in definitive study:	2, 4, and 8 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	5 mL/kg; cyclophosphamide at 10 mL/kg
Formulation/Vehicle:	Sterile water for injection, diluted with 55 mM citrate buffer and 3% (w:w) glycine, pH 5.8
Species/Strain:	CD-1 mice
Number/Sex/Group:	6 male mice/group at 2 and 4 mg/kg, and 14 male mice at 8 mg/kg
Satellite groups:	2 preliminary dose finding studies; groups of 3 mice/sex/dose
Basis of dose selection:	Dose selection for the main micronucleus assay was based on the range finding study.
Negative control:	Vehicle: sterile water for injection, diluted with 55 mM citrate buffer and 3% (w:w) glycine, pH 5.8
Positive control:	Cyclophosphamide monohydrate (CPS) 60 mg/kg administered once on Day 0

Table 43. Study design for in vivo clastogenic micronucleus assay in mice

Group	Treatment	Dose Level (mg/kg)	Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals with Bone Marrow Harvested[a]
					Males
1	Vehicle	0	0	5	10
2	MLN9708	2	0.4	5	5
3	MLN9708	4	0.8	5	5
4	MLN9708	8	1.6	5	10
5	Positive Control	60	6	10	5

[a] The first five (5) surviving animals in the Micronucleus groups (Groups 1-5) were utilized for bone marrow collection between 24 and 28 hours after dosing and the next five (5) surviving animals in the Micronucleus groups (Groups 1 and 4) were utilized for bone marrow collection between 44 and 48 hours after dosing

(Excerpted from the submission)

Analysis

- For the range finding study, groups of 3 mice/sex/dose were administered MLN9708 by oral gavage, single dose at 10, 20, and 30 mg/kg. All 3 doses caused severe toxicities and mortality. A second range-finding study was conducted at doses of 5, 6, and 8 mg/kg with 3 male mice/group. The majority of animals tolerated 8 mg/kg in the second range finding study (1/3 mortalities at 8 mg/kg).

- All mice were sacrificed 24 hours after receiving their final dose and femoral bone marrow smears prepared. Smears were stained with acridine orange prior to analysis with fluorescence microscopy to determine the proportion of polychromatic erythrocytes in the total erythrocyte count (% PCE) and the number of micronucleated polychromatic erythrocytes (MPCE) per 2000 PCE analysed.

Criteria for Positive Response
The test article may be considered positive for induction of micronuclei if a dose-related increase in the percentage of PCEs with micronuclei is observed and a statistically significant increase relative to the vehicle is seen at one or more dose levels.

Study Validity

The vehicle group mean must lie within the historical control. The positive control response must be higher than the vehicle group and be consistent with historical positive control data.

The toxicokinetic assessments (study number 13-039/tk) in a single dose study of MLN9708 for injection at 2, and 4 mg/kg in mice demonstrated systemic exposure, dosing appeared to be adequate. The Cmax and $AUC_{0\text{-}24h}$ values of MLN2238 increased dose-dependently when MLN9708 was administered orally to mice at 2 and 4 mg/kg as MLN2238. In the 8 mg/kg group, a decrease in locomotor activity and hypothermia were observed in all 5 animals, and all animals in this group were moribund and euthanized at 6 hours after dosing. The Cmax and $AUC_{0\text{-}24h}$ values were not calculated from 8 mg/kg group.

Results

- One male in the 8 mg/kg dose group was found dead on Day 2. Clinical signs included the body was cool to touch on Day 1; no significant macroscopic changes were observed. A maximum tolerated doses (MTD) was achieved (6 mg/kg) based clinical signs of toxicity and death.
- MLN-9708 did not produce an increase in the percent mean number of micronucleated polychromatic erythrocytes (%MN-PCEs) compared to the vehicle control group.
- Bone marrow cytotoxicity (decreases in the ratio of polychromatic to total erythrocytes, PCE:TE ratio) was not observed any of the treatment groups.

Reference ID: 3844453

Table 44. Summary evaluation of definitive phase bone marrow micronucleus assay in mice

Test Article	Dose (mg/kg)	No. of males used for micronucleus evaluation	Mean % MN PCEs (± SD)		Mean PCE:TE Ratio (± SD)	
			24 to 28 hours PD	44 to 48 hours PD	24 to 28 hours PD	44 to 48 hours PD
Vehicle	0	10	0.05 ± 0.05	0.03 ± 0.04	0.61 ± 0.04	0.62 ± 0.06
MLN9708	2	5	0.07 ± 0.03	N/A	0.63 ± 0.05	N/A
MLN9708	4	5	0.11 ± 0.13	N/A	0.69 ± 0.07	N/A
MLN9708	8	10	0.15 ± 0.08	0.09 ± 0.10	0.67 ± 0.08	0.54 ± 0.06
CPS	60	5	0.89 ± 0.35*	N/A	0.57 ± 0.04	N/A

* = $p<0.05$ when compared to the control group.
Abbreviations: No. = Number; MN = Micronucleated; PCE = Polychromatic erythrocytes; NCE = Normochromatic erythrocytes; TE = Total erythrocytes; SD = Standard Deviation; CPS = Cyclophosphamide monohydrate; N/A = Not applicable; PD = Post-dosing

(Excerpted from the submission)

Table 45. Individual and mean data for definitive phase bone marrow micronucleus assay in mice 24-48 hours after dosing

INDIVIDUAL AND MEAN DATA
Males, Between 24 and 28 Hours after Dosing Once

TREATMENT	ANIMAL No.	MN PCEs/ 2000 PCEs	% MN PCEs	PCEs	NCEs	PCE:TE Ratio
Vehicle	71518	1	0.05	601	399	0.60
	71519	0	0.00	637	363	0.64
	71521	2	0.10	558	442	0.56
	71522	2	0.10	645	355	0.65
	71524	0	0.00	587	413	0.59
Mean ± SD			**0.05 ± 0.05**			**0.61 ± 0.04**
MLN9708 (2 mg/kg)	71528	1	0.05	669	331	0.67
	71533	2	0.10	633	367	0.63
	71537	1	0.05	617	383	0.62
	71549	2	0.10	553	447	0.55
	71560	1	0.05	675	325	0.68
Mean ± SD			**0.07 ± 0.03**			**0.63 ± 0.05**
MLN9708 (4 mg/kg)	71525	1	0.05	619	381	0.62
	71532	0	0.00	742	258	0.74
	71536	6	0.30	741	259	0.74
	71538	4	0.20	745	255	0.75
	71555	0	0.00	610	390	0.61
Mean ± SD			**0.11 ± 0.13**			**0.69 ± 0.07**
MLN9708 (8 mg/kg)	71515	5	0.25	707	293	0.71
	71517	1	0.05	752	248	0.75
	71520	3	0.15	664	336	0.66
	71531	2	0.10	539	461	0.54
	71534	4	0.20	683	317	0.68
Mean ± SD			**0.15 ± 0.08**			**0.67 ± 0.08**
Cyclophosphamide (60 mg/kg)	71527	29	1.45	629	371	0.63
	71550	20	1.00	599	401	0.60
	71552	15	0.75	519	481	0.52
	71556	13	0.65	554	446	0.55
	71558	12	0.60	541	459	0.54
Mean ± SD			**0.89 ± 0.35***			**0.57 ± 0.04**

MN = Micronucleated
NCE = Normochromatic Erythrocyte
TE = Total erythrocytes (PCE + NCE)
PCE = Polychromatic Erythrocyte
Vehicle = 55 mM citrate buffer and 3% (w/w) glycine, pH 5.8
*Statistically different than vehicle control $p \leq 0.05$

(Excerpted from the submission)

Table 46. Individual and mean data for definitive phase bone marrow micronucleus assay in mice 44-48 hours after dosing

Males, Between 44 and 48 Hours after Dosing Once

TREATMENT	ANIMAL No.	MN PCEs/ 2000 PCEs	% MN PCEs	PCEs	NCEs	PCE:TE Ratio
Vehicle	71526	0	0.00	635	365	0.64
	71529	0	0.00	632	368	0.63
	71530	0	0.00	520	480	0.52
	71539	1	0.05	650	350	0.65
	71540	2	0.10	652	348	0.65
Mean ± SD			**0.03 ± 0.04**			**0.62 ± 0.06**
MLN9708 (8 mg/kg)	71543	1	0.05	452	548	0.45
	71544	1	0.05	525	475	0.53
	71546	5	0.25	574	426	0.57
	71547	2	0.10	519	481	0.52
	71548	0	0.00	609	391	0.61
Mean ± SD			**0.09 ± 0.10**			**0.54 ± 0.06**

MN = Micronucleated
NCE = Normochromatic Erythrocyte
TE = Total erythrocytes (PCE + NCE)
PCE = Polychromatic Erythrocyte
Vehicle = 55 mM citrate buffer and 3% (w/w) glycine, pH 5.8

7.4 Other Genetic Toxicity Studies

Study title: Comet assay of MLN-9708 for injection in mice

Study no:	B130357
Study report location:	4.2.3.3.2
Conducting laboratory:	Takeda Pharmaceutical Company Limited. Sunayama, Japan
Date of study initiation:	July 10, 2013
GLP compliance:	Signed and Included
QA statement:	Signed and Included
Drug, lot #, and % purity:	MLN-9708, Lot #21E004, 99.0% pure

Key Study Findings:

- MNL9708 was negative in the Comet assay in the liver and glandular stomach, under the conditions tested.

Reference ID: 3844453

Methods

Doses in definitive study:	0.5, 1, 2, 4 and 6 mg/kg/day
Frequency of dosing:	Once daily for 2 consecutive days
Route of administration:	Oral gavage
Dose volume:	8 mL/kg; 10 mL/kg positive control Ethyl methanesulfonate (EMS)
Formulation/Vehicle:	55 mmol/L citrate buffer and 3% (w/w) glycine, pH 5.8
Dose formulation analysis:	Stability was demonstrated and drug concentrations were within the acceptable range of the nominal concentrations
Species/Strain:	CD-1 mice
Number/Sex/Group:	5 males/group
Negative control:	55 mmol/L citrate buffer and 3% (w/w) glycine, pH 5.8 (vehicle)
Positive control:	Ethyl methanesulfonate (200 mg/kg)
Tissues to be analyzed:	Liver and glandular stomach cells
Sampling time(s):	Livers and glandular stomach cells were collected 3 hours after the last dose administration (24 hours after the first dose administration)
Histopathology:	Liver and stomach samples from the same portion of the tissue as used for the Comet assay were fixed and stored for possible histopathology examination
DNA staining method:	(b) (4)
Scoring method:	Fluorescence microscopy (image analysis)

Table 47. Study design for comet assay of MLN-9708 in mice

The experimental design in the comet assay was as follows.

Treatment group	Dose level * (mg/kg/day)	Dose volume (mL/kg)	Dose frequency	Sampling time	Number of animals / Animal number
Negative control (Vehicle)	0	8	2	3 hours after the final dosing	5 10101 - 10105
Test substance (MLN9708)	0.5				5 10201 - 10205
	1				5 10301 - 10305
	2				5 10401 - 10405
	4				5 10501 - 10505
	6				5 10601 - 10605
Positive control (EMS)	200	10			5 10701 - 10705

*: as MLN2238

(Excerpted from the submission)

Analysis

Each animal was anesthetized and euthanized to remove liver and stomach. Part of liver and stomach was used to prepare single cell suspensions to comet assay and the remaining parts of the tissues were processed for histopathological examinations. The slides specimens were processed for cell lysing, unwinding of DNA, electrophoresis for 20 minutes and neutralization. The slides were stained with staining solution (b) (4) and covered with a cover glass.

Comets of the cell nuclei were measured under a fluorescent microscope. For each animal, 100 cellular nuclei (50 cellular nuclei/slide) were analyzed for % tail DNA. Heavily damaged cells, commonly referred to as “hedgehogs” and consisting of small or non-existent head and largely diffused tails, were excluded from the data collection.

Study Validity

Individual values in the negative control group ranged within the historical control data (mean ± 3SD) at the test facility.
The mean value obtained from the positive control group was significantly higher than that from the negative control group statistically.

Criteria for Positive Response

The comet assay was judged positive if the test substance caused a significant increase in the % tail DNA not caused by the cytotoxic effects.

Results

No statistical significances were observed in the mean % tail DNA between the test substance groups and the negative control group for both liver and the glandular stomach. No abnormal necropsy findings were observed in any treated groups in the liver and the glandular stomach.

Reference ID: 3844453

Table 48. Summary results from comet assay in liver tissue from MLN-9708 testing in mice

Treatment group	Dose level [a] (mg/kg/day) Route Frequency	Animal number	% tail DNA [b] (Mean ± SD)	Incidence of [b] hedgehogs (%)
Negative control (Vehicle)	0	10101	2.92 ± 8.52	1.0
	p.o.	10102	2.26 ± 7.28	0.0
	Twice [c]	10103	2.62 ± 7.58	0.0
		10104	2.79 ± 6.66	0.0
		10105	3.33 ± 7.93	2.0
		Mean ± SD	2.78 ± 0.39	0.60 ± 0.89
Test substance (MLN9708)	2	10401	2.85 ± 7.20	0.0
	p.o.	10402	2.80 ± 7.03	0.0
	Twice [c]	10403	2.41 ± 5.71	1.0
		10404	2.95 ± 7.84	0.0
		10405	2.66 ± 6.16	0.0
		Mean ± SD	2.73 ± 0.21	0.20 ± 0.45
	4	10501	2.51 ± 6.17	0.0
	p.o.	10502	2.94 ± 6.86	0.0
	Twice [c]	10503	3.05 ± 8.33	1.0
		10504	2.65 ± 6.34	0.0
		10505	2.63 ± 7.08	1.0
		Mean ± SD	2.76 ± 0.23	0.40 ± 0.55
	6	10601	3.16 ± 6.17	0.0
	p.o.	10602	2.85 ± 7.26	1.0
	Twice [c]	10603	2.62 ± 5.84	2.0
		10604	2.24 ± 6.25	0.0
		10605	2.25 ± 7.28	1.0
		Mean ± SD	2.62 ± 0.40	0.80 ± 0.84
Positive control (EMS)	200	10701	18.26 ± 7.50	0.0
	p.o.	10702	17.58 ± 8.87	1.0
	Twice [c]	10703	17.60 ± 7.32	1.0
		10704	16.20 ± 7.79	0.0
		10705	12.85 ± 6.15	1.0
		Mean ± SD	16.50 ± 2.17 **	0.60 ± 0.55

Vehicle: 55 mmol/L citrate buffer and 3% (w/w) glycine, pH5.8

a) As MLN2238, b) Calculated from 100 cellular nuclei per animal,

c) Administered 24 and 3 hours before sampling

**: Significantly ($p \leq 0.01$) different from the negative control group by Student's *t*-test

(Excerpted from the submission)

Table 49. Summary results from comet assay in stomach tissue from MLN-9708 testing in mice

Treatment group	Dose level [a] (mg/kg/day) Route Frequency	Animal number	% tail DNA (%) [b] (Mean ± SD)	Incidence of [b] hedgehogs (%)
Negative control (Vehicle)	0 p.o. Twice [c]	10101	4.85 ± 10.52	2.0
		10102	4.87 ± 9.49	1.0
		10103	4.54 ± 8.98	1.0
		10104	5.07 ± 8.85	1.0
		10105	4.75 ± 9.23	0.0
		Mean ± SD	4.82 ± 0.19	1.00 ± 0.71
Test substance (MLN9708)	2 p.o. Twice [c]	10401	4.47 ± 8.79	2.0
		10402	5.15 ± 9.00	2.0
		10403	4.54 ± 9.65	0.0
		10404	5.24 ± 11.00	0.0
		10405	3.58 ± 8.68	0.0
		Mean ± SD	4.60 ± 0.67	0.80 ± 1.10
	4 p.o. Twice [c]	10501	4.51 ± 9.39	0.0
		10502	4.72 ± 7.90	3.0
		10503	4.77 ± 8.98	0.0
		10504	4.60 ± 8.82	1.0
		10505	4.63 ± 8.02	0.0
		Mean ± SD	4.65 ± 0.10	0.80 ± 1.30
	6 p.o. Twice [c]	10601	5.76 ± 9.39	0.0
		10602	4.41 ± 8.53	1.0
		10603	4.36 ± 9.38	1.0
		10604	4.83 ± 9.05	0.0
		10605	4.71 ± 9.38	1.0
		Mean ± SD	4.81 ± 0.56	0.60 ± 0.55
Positive control (EMS)	200 p.o. Twice [c]	10701	21.18 ± 10.80	1.0
		10702	18.90 ± 11.35	0.0
		10703	18.25 ± 9.86	0.0
		10704	20.38 ± 9.48	0.0
		10705	17.49 ± 12.99	2.0
		Mean ± SD	19.24 ± 1.52 **	0.60 ± 0.89

Vehicle: 55 mmol/L citrate buffer and 3% (w/w) glycine, pH5.8

a) As MLN2238, b) Calculated from 100 cellular nuclei per animal

c) Administered 24 and 3 hours before sampling

**: Significantly ($p \leq 0.01$) different from the negative control group by Student's *t*-test

(Excerpted from the submission)

Toxicokinetics

Table 50. Summary toxicokinetics following single oral dose of MLN-9708 for injection in mice (Study 13-039/tk)

Dose (mg/kg)		Concentration (ng/mL) 0.25 h	0.5 h	1 h	6 h	24 h	AUC 0-24h (ng·h/mL)	Cmax (ng/mL)	Tmax (h)
2		79	90	136	14	5			
		103	89	80	12	6			
	Mean	91	90	108	13	6	557	108	1.0
4		224	262	165	22	5			
		213	171	193	22	5			
	Mean	219	217	179	22	5	926	219	0.3
8		1008	729	527	54	-			
		652	545	644	47	-			
	Mean	830	637	586	51	N.C.	N.C.	N.C.	N.C.

-: No sample due to death
N.C.: Not calculated

(Excerpted from the submission)

8 Carcinogenicity

Studies not conducted.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Studies not conducted.

9.2 Embryonic Fetal Development

Study title: Range finding study for effects of MLN9708 on Embryo-fetal development in rats

Study no.:	13-07/te
Study report location:	4.2.3.5.2
Conducting laboratory and location:	Takeda Pharmaceutical Company Limited, Kanagawa, Japan
Date of study initiation:	September 26, 2013
GLP compliance:	Non GLP
QA statement:	Not included
Drug, lot #, and % purity:	MLN9708; lot # 101296; 69.3%

Reference ID: 3844453

Key Study Findings

- Three maternal mortalities observed at 0.8 mg/kg/3 days (3/6 in this dose group). All other dams survived the duration of the study cycle.
- Fetal malformations at 0.6 mg/kg/3 days include increased percentage of biparate ossification of the thoracic centrum, and shortened 13th rib. Upon daily administration at 0.3 mg/kg/day fetal malformations included left umbilical artery, malpositioned subclavian branch, malpositioned common carotid artery, and retroesophageal aortic arch.
- Maternal toxicity and embryo-fetal effects were observed at ≥0.6 mg/kg (Gestation Day 19 $AUC_{0\text{-}72hr}$ of 1081 hr*ng/mL) and included post implantation loss, decreased fetal weight, and reduced fetal viability.

Methods

Doses:	0.4, 0.6, 0.8 mg/kg every 3 days (4 total doses); 0.1 and 0.3 mg/kg/daily
Frequency of dosing:	Every 3 days (Days 6, 9, 12 and 15 of gestation); and once daily for 0.1 and 0.3 mg/kg dose groups (Day 6-17 of gestation).
Dose volume:	5mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	55-mM citrate buffer containing 0.45 weight/volume percent (w/v %) NaCl in sterile water for injection
Species/Strain:	Crl:CD Sprague Dawley
Number/Sex/Group:	6/group
Satellite groups:	3 animals per dose group were used for toxicokinetics.
Study design:	N/A
Deviation from study protocol:	N/A

Observations and Results

Mortality

At 0.8 mg/kg/3 days 3 animals died (one found dead #5F03, two sacrificed moribund # 5F01, 5F02) on Day 7. They presented with the following clinical signs: decreased food consumption, locomotor activity, and hypothermia. Gross pathology findings included: pale liver and stomach distention in one animal sacrificed moribund (# 5F02). One of the sacrificed animals was not pregnant (#5F01). Due to the severe maternal toxicity at 0.8 mg/kg, 3 non-treated animals in this group were reassigned to 0.4 mg/kg/3days dose group.

Clinical Signs

Additional clinical signs observed at 0.6 mg/kg/3 days included: soiled fur, decreased feces, abnormal gait, and sanguineous discharge from vagina. At 0.3 mg/kg/ day decreased feces and black stool was reported.

Reference ID: 3844453

Body Weight

Body weight gain was statistically significantly reduced at 0.6 mg/kg/3 days from Day 8 throughout the treatment and post treatment recovery period (Gestational Day 20). Body weight gain was also reduced significantly in the 0.3 mg/kg/day dose group from Day 8 through the post treatment period (Gestational Day 18).

Feed Consumption

Food consumption was statistically significantly reduced at 0.6 mg/kg/3 days from Day 6 throughout the treatment period (Gestational Day 16). Food consumption was also reduced significantly in the 0.3 mg/kg/day dose group on Gestational Day 6 and Day 12.

Toxicokinetics

Table 51. Toxicokinetic parameters in embryonic fetal development dose range finding toxicity study in rats (daily administration)

Concentrations of MLN2238 in female rat plasma (Daily administration groups)

Dose (mg/kg/day)	Animal No.	Concentration (ng/mL) Pre	1 h	2 h	4 h	8 h	24 h	AUC_{0-24h} (ng·h/mL)	Cmax (ng/mL)	Tmax (h)
0.1	6F001	6	9	10	9	7	8	188	10	2.0
	6F002	6	9	9	9	7	7	179	9	1.0
	Mean	6	9	10	9	7	8	184	10	1.5
0.3	7F001	10	39	28	19	14	11	371	39	1.0
	7F002	9	19	16	16	14	11	324	19	1.0
	Mean	10	29	22	18	14	11	348	29	1.0

Pre: Before dosing

(Excerpted from the submission)

Table 52. Toxicokinetic parameters in embryonic fetal development dose range finding toxicity study in rats (administration/ 3 days)

Concentrations of MLN2238 in female rat plasma (Intermittent administration groups)

Dose (mg/kg/day)	Animal No.	Concentration (ng/mL) Pre	1 h	2 h	4 h	8 h	24 h	72 h	AUC_{0-24h} (ng·h/mL)	AUC_{0-72h} (ng·h/mL)	Cmax (ng/mL)	Tmax (h)
0.4	9F001	5	15	14	12	11	11	6	273	681	15	1.0
	9F002	6	27	23	17	12	9	4	308	620	27	1.0
	Mean	6	21	19	15	12	10	5	291	651	21	1.0
0.6	8F001	6	59	49	24	18	14	6	500	980	59	1.0
	8F002	6	74	52	34	22	16	8	605	1181	74	1.0
	Mean	6	67	51	29	20	15	7	553	1081	67	1.0

Pre: Before dosing

(Excerpted from the submission)

Dosing Solution Analysis

The mean concentrations of MLN9708 in solution at 0.002 w/v% after storage for 11 days in a refrigerator (control range: 2°C to 8°C)and for 24 hours at room temperature was measured and these results were within acceptability criteria of ±10% of nominal concentrations, indicating accurate formulation. Given the results, dosing solution for the study was prepared at once and stored in a refrigerator. The concentrations of

MLN2238 in the 0.002, 0.006, .008 and 0.012 w/v% dosing solutions of MLN9708 were assayed three times, during the dosing period. The concentrations in the solutions were reported to be within the acceptability criteria ± 10%.

Table 53. Dosing solution stability analysis in a embryofetal toxicity dose range finding study in rats

Target conc.[a] (w/v%)	Storage period	Found (w/v%)		Found/ Target[b] (%)	Stability[c] (%)
		Each value	Mean		
0.002	Initial	0.00195 0.00195 0.00198	0.00196	98	-
	After 11 days in a cold place[d] and 24 hours at room temperature[e]	0.00198 0.00199 0.00201	0.00199	-	102

(Excerpted from the submission)

Table 54. Dosing solution concentration analysis in a embryofetal toxicity dose range finding study in rats

Target conc. (w/v%)	Found (w/v%)		Found/ Target [a] (%)
	Each value	Mean	
0.002	0.00202 0.00202 0.00202	0.00202	101
0.006	0.00554 0.00587 0.00575	0.00572	95
0.008	0.00803 0.00796 0.00800	0.00800	100
0.012	0.0120 0.0120 0.0120	0.0120	100

(Excerpted from the submission)

Necropsy

Gross pathology was in the dams was unremarkable with the exception of the pale liver and stomach distention in one animal sacrificed moribund (# 5F02) on Day 7.

Table 55. Placental gross pathology findings in rats

Macroscopic findings- Daily administration	Placental findings		
Dose (mg/kg/day)	0	0.1	0.3
No. placenta	91	74	80
Enlarged Placenta (% frequency)	-	3.7	-

(-): no change/no toxicologically significant change

Gross pathology in the placenta was unremarkable for dose groups with intermittent dosing (up to 0.6 mg/kg/ 3 days).

Cesarean Section Data

Table 56. Pregnancy parameters in an embryofetal toxicity dose range finding study in rats

Dose (mg/kg/3days)	0	0.4	0.6
No. Dams	3	3	3
Corpora lutea	16.8	16.7	15.8
Implantation sites	15.5	16.0	14.2
Live fetuses	15.2	15.7	10.7
Dead fetuses	0	0	0
Total resorptions	0	0	0
Pre-implantation loss	7.1	4.2	7.9
Post-implantation loss	2.1	2.4	25.2
Mean fetus weight (g)	3.6/3.76	3.47/3.54	3.02*/3.20*

Mean fetus weight (g) given in female/male
*p<0.05

Dose (mg/kg/day)	0	0.1	0.3
No. Dams	3	3	3
Corpora lutea	16.8	15.5	15.8
Implantation sites	15.5	13.2	14.5
Live fetuses	15.2	12.3*	13.3*
Dead fetuses	0	0	0
Total resorptions	0	0	0
Pre-implantation loss	7.1	15.6	8.3
Post-implantation loss	2.1	5.6	7.8
Mean fetus weight (g)	3.6/3.76	3.38/3.67	3.24/3.49

Mean fetus weight (g) given in female/male
*p<0.05

Reference ID: 3844453

Offspring

Table 57. Fetal malformations, abnormalities, and variations in dose range finding study in rats

		Litter		
Dose (mg/kg)		0	0.4	0.6
External Abnormalities				
Numbers Examined -*External*		49	N/A	34
Tongue	Ankyloglossia	-	-	1.4
Minor Visceral Abnormalities				
Numbers Examined		49	N/A	34
Heart	Membranous ventricular septal defect	2.1	N/A	2.2
Minor Visceral Variations				
Numbers Examined		49	N/A	34
Artery	Left umbilical artery	1.9	N/A	0
Minor Skeletal Variations				
Numbers Examined		49	N/A	34
Vertebral column	Bipartite ossification thoracic centrum	4.9	N/A	12.9
Ribs	Short supernumerary rib	8.8	N/A	0
	Short 13th rib	0	N/A	5.0

(-): no change/no toxicologically significant change
NA: not applicable; data not reported

Daily administration		Litter		
Dose (mg/kg)		0	0.1	0.3
Minor Visceral Abnormalities				
Numbers Examined				
Eye	Microphthalmia	-	N/A	2.1
Heart	Ventricular Septal Defect	2.1	N/A	2.1
	Malpositioned subclavian branch	-	N/A	2.1
	Malpositioned common carotid artery	-	N/A	2.1
	Retroesophageal aortic arch	-	N/A	2.1
Minor Visceral Variations				
Numbers Examined				
Artery	Left Umbilical Artery	1.9	N/A	6.3
Minor Skeletal Variations				
Vertebral column	Bipartite ossification thoracic centrum	4.9	N/A	2.8
Ribs	Short supernumerary rib	8.6	N/A	8.3

Reference ID: 3844453

Study title: Effects of MLN 9708 on Embryo-fetal development in Rats

Study no.:	13-155/TE
Study report location:	4.2.3.5.2
Conducting laboratory and location:	Takeda Pharmaceutical Company Limited, Kanagawa, Japan
Date of study initiation:	February 24, 2014
GLP compliance:	Signed and Included; Japan
QA statement:	Signed and Included
Drug, lot #, and % purity:	MLN9708; lot # 102031; 99.3%

Key Study Findings

- Two maternal mortalities at 0.6 mg/kg/3 days (2/20 in this dose group). All other dams survived the duration of the study cycle.
- A slight but not statistically increase in post implantation loss was observed at 0.6 mg/kg/3 days.
- Maternal toxicity was observed at ≥0.6 mg/kg (Gestation Day 15 $AUC_{0\text{-}72hr}$ of 1103 hr*ng/mL).

Methods

Doses:	0.2, 0.4, 0.6 mg/kg every 3 days (4 total doses)
Frequency of dosing:	Every 3 days (Days 6, 9, 12 and 15 of gestation)
Dose volume:	5mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	55-mM citrate buffer containing 0.45 weight/volume percent (w/v%) NaCl in sterile water for injection
Species/Strain:	Crl:CD Sprague Dawley
Number/Sex/Group:	20/group
Satellite groups:	Groups 5-8 were used for toxicokinetics (see Table 58).
Study design:	N/A
Deviation from study protocol:	N/A

Reference ID: 3844453

Table 58. Study design for embryonic fetal development toxicity study in rats

The groups were as follows.

Group	Test article	Dosage level [a)] (mg/kg/3 days)	Dosage volume (mL/kg/3 days)	Concentration [a)] (w/v%)	Number of animals (Animal number)
1	Control	0	5	0	20 (1F01-20)
2	MLN9708	0.2	5	0.004	20 (2F01-20)
3	MLN9708	0.4	5	0.008	19 (3F01-13, 15-20) [c)]
4	MLN9708	0.6	5	0.012	20 (4F01-20)
5 [b)]	Control	0	5	0	4 (5F01-04)
6 [b)]	MLN9708	0.2	5	0.004	4 (6F01-04)
7 [b)]	MLN9708	0.4	5	0.008	4 (7F01-04)
8 [b)]	MLN9708	0.6	5	0.012	4 (8F01-04)

Control: 55 mM/L citrate buffer containing 0.45 w/v% NaCl, pH5.8
a) As MLN2238. b) Satellite groups for toxicokinetics.
c) Animal 3F14 was not pregnant.

(Excerpted from the submission)

Groups 5 to 8 were used only for toxicokinetics.

Based on results from the preliminary dose range finding study, the high dose group was set at 0.6 mg/kg/dosing every 3 days, which should induce both maternal and embryo-fetal toxicities and the middle and low dose groups were set at 0.4 and 0.2 mg/kg/dosing every 3 days, respectively.

Observations and Results

Mortality

Table 59. Cause of maternal death in embryonic fetal development toxicity study in rats

Animal #	Dose (mg/kg/3 days)	Day of Death	Observations	
			Reason	General (include pathology)
4F10	0.6	7	Found dead	Soiled fur on abdomen and vulva, stomach discoloration black
4F11	0.6	8	Found dead	No significant clinical signs or pathology

Clinical Signs

All animals were observed for viability and clinical signs twice a day (before and 0.5 to 1.5 hours after dosing) on the dosing days and once a day on the non-dosing days. In the 0.6-mg/kg/3 days group, three dams had reported decreased feces (Animals 4F01, 4F02 and 4F20) and two dams had reported soiled fur (Animals 4F10 and 4F20).

Body Weight

All dams were weighed on Days 0, 6, 8, 9, 11, 12, 14, 15, 17, and 20 of gestation. Body weight gain was statistically significantly reduced on Days 8, 11, 14 and 17 of gestation for animals dosed at 0.2 mg/kg/3 days, and on Day 11 of gestation for animals dosed at

0.4 mg/kg/3days. Animals dosed at 0.6 mg/kg/3days group showed decreased body weight gain on Days 8, 11, 14, and 17 of gestation and increased on Days 12 and 15 of gestation.

Feed Consumption

24-hour food consumption was measured on Days 0, 6, 8, 9, 11, 12, 14, 15, 17, and 19 of gestation. There were statistically significant decreases in food consumption on Days 6, 9, 12, and 15 of gestation in the 0.2 mg/kg/3days and 0.4 mg/kg/3days groups. There were statistically significant decreases in food consumption between Days 6 and 9, and Days 12 and 15 of gestation in the 0.6 mg/kg/3days group.

Toxicokinetics

Blood samples (0.3 mL) were collected from the jugular vein of 4 animals in each satellite group (See Table 58) with heparinized syringes at 1, 2, 4, 8, 24, and 72 hours after dosing on the first and last day of dosing (Days 6 and 15 of gestation) and before dosing on the last dosing day (Day 15).
Increases in the C_{max} of MLN9708 were greater than dose proportional to the increases in the doses between 0.2 and 0.6 mg/kg/3days. Increases in the AUC of MLN9708 were proportional to the increases in the doses between 0.2 and 0.6 mg/kg/3days.

Table 60. Toxicokinetic parameters in embryonic fetal development toxicity study in rats

Summary of Mean Toxicokinetic Parameters of MLN2238 in Plasma After Oral Administration of MLN9708 to Pregnant Rats

Dose (mg/kg/3 days)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng·h/mL)	AUC_{0-72h} (ng·h/mL)
Day 6 of gestation				
0.2	1.7	6	99	275
0.4	1.0	22	246	590
0.6	1.0	46	428	964
Day 15 of gestation				
0.2	2.0	12	201	489
0.4	1.0	33	346	738
0.6	1.0	49	511	1103

AUC_{0-24h} = area under the concentration-time curve (AUC) from the time 0 to 24 hours; AUC_{0-72h} = AUC from the time 0 to 72 hours; C_{max} = maximum observed plasma concentration; T_{max} = time to reach C_{max}.

Dose (mg/kg/3 days)	C_{max}/Dose (ng/mL/mg/kg)	AUC_{0-24h}/Dose (ng·h/mL/mg/kg)	AUC_{0-72h}/Dose (ng·h/mL/mg/kg)
Day 6 of gestation			
0.2	30	495	1375
0.4	55	615	1475
0.6	77	713	1607
Day 15 of gestation			
0.2	60	1005	2445
0.4	83	865	1845
0.6	82	852	1838

(Excerpted from the submission)

Dosing Solution Analysis

Concentration and Stability

The mean concentrations of MLN9708 in solution at 0.004, 0.008, and 0.012 w/v% after storage for 10 days in a refrigerator (control range: 2°C to 8°C)and for 24 hours at room temperature was measured and these results were within acceptability criteria of ±10% of nominal concentrations, indicating accurate formulation. Given the results, dosing solution for the study was prepared at once and stored in a refrigerator. The concentrations of MLN2238 in the 0.004, 0.008, and 0.012 w/v% dosing solutions of MLN9708 were assayed twice, on the first and the last day of preparation, during the

dosing period. The concentrations in the solutions were reported to be within the acceptability criteria ± 10%.

Necropsy

Table 61. Maternal gross pathology findings in rats

Macroscopic findings		Dams			
Dose (mg/kg/3days)		0	0.2	0.4	0.6
No. animals		20	20	19	20
Thymus	small	-	-	1	11
Stomach	Dark discoloration	-	-	-	1

(-): no change/no toxicologically significant change

Table 62. Placental gross pathology findings in rats

Macroscopic findings		Placental findings			
Dose (mg/kg/3days)		0	0.2	0.4	0.6
No. animals		20	20	19	20
Placenta	Fused placenta	-	-	1	-

(-): no change/no toxicologically significant change

Cesarean Section Data

Table 63. Pregnancy parameters in rats

Dose (mg/kg/3days)	0	0.2	0.4	0.6
No. Dams	20	20	19	20
Corpora lutea	17.1	15.8	15.9	16.2
Implantation sites	15.5	14.9	15.2	14.8
Live fetuses	14.8	14.2	14.6	13.7
Dead fetuses	0.1	0.0	0.0	0.1
Total resorptions	0.0	0.0	0.0	0.0
Pre-implantation loss	9.5	5.4	3.5	8.9
Post-implantation loss	0.5	0.8	0.6	1.1
Mean fetus weight (g)	3.5/3.67	3.51/3.68	3.44/3.71	3.35/3.53

Mean fetus weight (g) given in female/male

Reference ID: 3844453

Offspring

Table 64. Fetal malformations, abnormalities, and variations in rats

		Litter			
Dose (mg/kg)		0	0.2	0.4	0.6
Major Malformations					
Numbers Examined -*External*		295	283	278	246
Head	Domed head	3	1	-	
	Cleft palate	3	-	-	
	Macroglossia	3	-	-	
	Mandibular micrognathia	3	-	-	-
	Maxillary micrognathia	3	-	-	-
Skin, tissue	Anasarca	1	-	-	-
Neural tube	Meningocele	-	1	-	-
GI	Umbilical hernia	-	-	1	-
Heart	Membranous ventricular septal defect	1	-	-	1
Minor Visceral Anomalies					
Numbers Examined					
Artery	Left umbilical artery	4	NA	NA	-
Adrenal gland	discolored	1	NA	NA	-
Minor Skeletal Variations and Anomalies					
Numbers Examined					
mandible	small	2	NA	NA	-
Vertebral column	Supernumerary lumbar vertebra	-	NA	NA	1
	Bipartite ossification thoracic centrum	7	NA	NA	1
	Bipartite ossification of lumbar centrum	3	NA	NA	2
Ribs	Short supernumerary rib	9	NA	NA	5

(-): no change/no toxicologically significant change
NA: not applicable; data not reported

Reference ID: 3844453

Study title: An Oral (Gavage) Dose Range-Finding Study of MLN9708 on Embryo/Fetal Development in Rabbits

Study no.:	(b) (4)-416156
Study report location:	4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 6, 2012
GLP compliance:	Signed and Included
QA statement:	Signed and Included
Drug, lot #, and % purity:	MLN9708; lot # 21E004; 99.0%

Key Study Findings

- Three maternal mortalities observed at 1.2 mg/kg/3 days (3/6 in this dose group). All other dams survived the duration of the study cycle.
- Fetal malformations at 1.2 mg/kg/3 days include omphalocele.
- Embryo-fetal effects were observed at 1.0 mg/kg (Gestation Day 19 $AUC_{0\text{-}72hr}$ of 1340 hr*ng/mL) and included post implantation loss, total resorptions, and reduced fetal viability; increased pre-implantation loss was observed at a dose of 0.5 mg/kg (Gestation Day 19 $AUC_{0\text{-}72hr}$ of 956 hr*ng/mL).

Methods

Doses:	0.25, 0.5, 1.0, 1.2 mg/kg/3 days
Frequency of dosing:	Every 3 days (Days 7,10,13, 16 and 19 of gestation)
Dose volume:	5mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	55-mmol/L citrate solution containing 3 weight/volume percent (w/v%) glycine
Species/Strain:	New Zealand White Rabbit
Number/Sex/Group:	6/group
Satellite groups:	N/A
Study design:	N/A
Deviation from study protocol:	N/A

Reference ID: 3844453

Table 65. Study design for dose range finding embryonic fetal development toxicity study in rabbits

Group	Test Article	Dose (mg/kg)	Dose Volume (mL/kg)	Number of Animals
1	Vehicle Control	0	5	6
2	MLN9708	0.25	5	6
3	MLN9708	0.5	5	6
4	MLN9708	1	5	6
5	MLN9708	1.2	5	6

(Excerpted from the submission)

Observations and Results

Mortality

One female (# 68522) in the 1.2 mg/kg group was found dead on Gestation Day 11; two females in the 1.2 mg/kg group (# 68517 and #68513) were sacrificed on Gestation Days 13 and 19, respectively, following body weight losses (16.4% and 17.7% of Gestation Day 7 body weight, respectively) and reduced food consumption. Additional clinical signs for these animals starting at Gestational Day 8 included decreased feces, diarrhea, soft stool, mucoid feces, and/or brown material on various body surfaces

Clinical Signs

Decreased feces, diarrhea, and/or soft stool were reported in the 0.5, 1.0, and 1.2 mg/kg dose groups starting on Gestational Day 8 up to Gestation Day 24.

Body Weight

Through the overall treatment period of Gestational Day 7 to 22, significantly decreased mean body weight gain was reported at 0.5 mg/kg dose group and a mean body weight loss was reported at 1.0 and 1.2 mg/kg groups.

Feed Consumption

Statistically significant decreased food consumption was reported during the entire treatment period (Gestational Day 7 to 22) in 1.0 and 1.2 mg/kg dose groups. Food consumption was also reported statistically significantly lower at 0.5 mg/kg dose groups from Gestational Day 7 to 13.

Toxicokinetics

Table 66. Summary toxicokinetics

Dose (mg/kg)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-72hr} (hr*ng/mL)
Gestation Day 7			
0.25	48	7.82	426
0.50	48	13.0	785
1.0	24	17.5	1070
1.2	8.0	23.8	1390
Gestation Day 19			
0.25	8.0	10.9	549
0.50	4.0	18.1	956
1.0	4.0	33.4	1340
1.2	4.0	66.6	1600

AUC_{0-72hr} = Area under the concentration-versus-time curve from 0 to 72 hours

C_{max} = Maximum concentration

T_{max} = Time to maximum concentration

(Excerpted from the submission)

Dosing Solution Analysis

The mean concentrations of MLN9708 in solution at 1.00 and 50.0 µg/mL after storage for 5 days in a refrigerator (control range: 2°C to 8°C) and for 17 hours at room temperature was measured and these results were within acceptability criteria of ±10% of nominal concentrations, indicating accurate formulation. Given the results, dosing solution for the study was prepared at once and stored in a refrigerator. The concentrations of MLN9708 in the 0.05, to 1.0 mg/mL dosing solutions were assayed three times, during the dosing period. The concentrations in the solutions were reported to be within the acceptability criteria ± 10%.

Table 67. Dosing solution concentration analysis in a embryofetal toxicity dose range finding study in rabbits

Date of Preparation	0.05 mg/mL	0.1 mg/mL	0.2 mg/mL	0.24 mg/mL
06 December 2012	0.0498 (99.5)	0.0985 (98.5)	0.199 (99.5)	0.239 (99.6)
09 December 2012	0.0500 (100)	0.0988 (98.8)	0.200 (100)	0.241 (100)
12 December 2012	0.0484 (96.7)	0.0952 (95.2)	0.192 (96.1)	0.228 (94.9)
15 December 2012	0.0468 (93.5)	0.0945 (94.5)	0.189 (94.7)	0.232 (96.8)
18 December 2012	0.0491 (98.1)	0.101 (101)	0.201 (100)	0.240 (99.9)

(Excerpted from the submission)

Necropsy

Two of the animals found dead had cystic oviducts reported upon necropsy (# 68517 Gestational Day13, #68513 Gestational Day 19). Additionally animal #68513 had an accessory spleen. Animal #68522 found dead on Gestational Day 11 was unremarkable upon necropsy. Gross pathology in the placenta was not reported.

Reference ID: 3844453

Table 68. Maternal gross pathology findings in a dose range finding study in rabbits

Macroscopic findings		Dams				
Dose (mg/kg/3days)		0	0.25	0.5	1.0	1.2
No. animals		5	6	5	6	6
Female - reproductive	Cystic Oviducts	1	1	1	-	2
Spleen	Accessory spleen	-	-	1	-	1
Lung	Dark areas			1		

(-): no change/no toxicologically significant change

Cesarean Section Data

Table 69. Pregnancy parameters in a dose range finding study in rabbits

Dose (mg/kg/3days)	0	0.25	0.5	1.0	1.2
No. Dams	5	6	5	6	3
Corpora lutea	50	62	53	60	27
Implantation sites	49	58	46	60	25
Live fetuses	47	56	45	51	24
Dead fetuses	0	0	0	0	0
Total resorptions	2	2	1	9	1
Pre-implantation loss	1	4	7	0	2
Post-implantation loss	2	2	1	9	1
Mean fetus weight (g)	41.0	41.5	41.6	39.1	41.4

Mean fetus weight (g) average for males and females combined

Offspring

Table 70. Fetal malformations, abnormalities, and variations in a dose range finding study in rabbits

		Litter				
Dose (mg/kg)		0	0.25	0.5	1.0	1.2
Major Malformations						
Numbers Examined *-External*						
Abdomen	Omphalocele	-	-	-	-	1

(-): no change/no toxicologically significant change

Study title: Effects of MLN 9708 on Embryo-fetal development in Rabbits

Study no.:	14-027/TE
Study report location:	4.2.3.5.2
Conducting laboratory and location:	Takeda Pharmaceutical Company Limited, Kanagawa, Japan
Date of study initiation:	February 24, 2014
GLP compliance:	Signed and Included; Japan
QA statement:	Signed and Included
Drug, lot #, and % purity:	MLN9708; lot # 102031; 99.3%

Key Study Findings

- There were no early mortalities in any dose group.
- Increased post implantation loss at ≥ 1.0 mg/kg/3 days.
- Fetal malformations at ≥ 0.3 mg/kg/3 days include caudal vertebrae, number of lumbar vertebrae, full supernumerary ribs, asymmetric vertebrae, shortened tail.
- Maternal toxicity and embryo-fetal effects were observed at ≥ 0.3 mg/kg (Gestation Day 19 AUC_{0-72hr} of 792 hr*ng/mL) and included fetal malformations.

Methods

Doses:	0.1, 0.3, 1.0 mg/kg every 3 days (4 total doses)
Frequency of dosing:	Every 3 days (Days 7,10,13, 16 and 19 of gestation)
Dose volume:	5mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	55-mmol/L citrate solution containing 3 weight/volume percent (w/v%) glycine
Species/Strain:	New Zealand White Rabbit
Number/Sex/Group:	18/group
Satellite groups:	N/A
Study design:	N/A
Deviation from study protocol:	N/A

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Table 71.Study design for embryonic fetal development toxicity study in rabbits

The dosage levels and experimental design were as follows.

Group	Test Article	Dosage Level [a] (mg/kg/3days)	Dosage Volume (mL/kg/3days)	Concentration [a] (weight/volume%)	Number of Animals Animal Number
1	Control	0	5	0	18 1F01, 1F03, 1F05-19
2	MLN9708	0.1	5	0.002	19 2F01-19
3	MLN9708	0.3	5	0.006	19 3F01-18, 20
4	MLN9708	1.0	5	0.02	19 4F01-09, 11-20

Control: 55-mmol/L citrate buffer containing 3 weight/volume% glycine, pH 5.8
a: As MLN2238.
Animals 1F04, 1F20, 2F20, 3F19, and 4F10 were not pregnant. Animal 1F02 was excluded from evaluation of the study because of death resulting from an intubation error. Litter data for Animals 1F16 and 2F15 were excluded from evaluation of the study because identification of the uterus for these dams was not conducted at necropsy and correct identification of the uterus and the fetuses for the dams could not be conducted.

(Excerpted from the submission)

Observations and Results

Mortality

No test article related deaths in any group.

Clinical Signs

Having decreased feces was reported in all dose groups and was showed a dose dependent effect (19/19 animals at 1.0 mg/kg, 13/19 animals at 0.3 mg/kg, 4/19 animals at 0.1 mg/kg/3days). At 1.0 mg/kg/3 days soiled fur was reported in 3/19 animals and loose stool were noted in 2/19 animals.

Body Weight

At 0.3 mg/kg/3 days and 1.0 mg/kg/3 days, body weight gain was decreased during Days 7 through 9 and Days 10 through 12 of gestation. Additionally at 1.0 mg/kg/3days body weight gains were decreased during Days 13 through 15 of gestation.

Feed Consumption

Food consumption was significantly decreased on dosing days (Days 7, 10, 13, 16, and 19) in the 0.3 mg/kg/3 days and 1.0-mg/kg/day/3days groups. Food consumption was only decreased on Day 19 in the 0.1 mg/kg/3 days group.

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Toxicokinetics

Table 72. Toxicokinetic parameters in embryonic fetal development toxicity study in rabbits

Summary of Mean Toxicokinetic Parameters of MLN2238 in Plasma After Oral Administration of MLN9708 to Pregnant Rabbits

Dose (mg/kg/3 days)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng·h/mL)	AUC_{0-72h} (ng·h/mL)
Day 7 of gestation				
0.1	32.0	3	30	162
0.3	32.0	11	191	679
1.0	5.3	22	443	1203
Day 19 of gestation				
0.1	4.0	9	189	497
0.3	8.0	15	324	792
1.0	6.7	29	586	1314

AUC_{0-24h} = area under the concentration-versus-time curve from 0 to 24 hours; AUC_{0-72h} = area under the concentration-versus-time curve from 0 to 72 hours; C_{max} = maximum concentration; T_{max} = time to maximum concentration.

Dose (mg/kg/3 days)	C_{max}/Dose (ng/mL/mg/kg)	AUC_{0-24h}/Dose (ng·h/mL/mg/kg)	AUC_{0-72h}/Dose (ng·h/mL/mg/kg)
Day 7 of gestation			
0.1	30	300	1620
0.3	37	637	2263
1.0	22	443	1203
Day 19 of gestation			
0.1	90	1890	4970
0.3	50	1080	2640
1.0	29	586	1314

(Excerpted from the submission)

Dosing Solution Analysis

The mean concentrations of MLN9708 in solution at 0.02 and .002 w/v% after storage for 10 days in a refrigerator (control range: 5.6°C to 6.7°C) and for 24 hours at room temperature (21.7°C to 22.9°C) was measured and these results were within acceptability criteria of ±10% of nominal concentrations, indicating accurate formulation. Given the results, dosing solution for the study was prepared at once and stored in a refrigerator. The concentrations of MLN9708 in 0.002, 0.006, and 0.02 w/v% dosing

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solutions were assayed two times, during the dosing period. The concentrations in the solutions were reported to be within the acceptability criteria ± 10%.

Table 73. Dosing solution concentration analysis in an embryofetal toxicity study in rabbits

Target conc. (w/v%)	Storage period	Found (w/v%)		Found/Target[a)] (%)	Stability[b)] (%)
		Each value	Mean		
0.002	Initial	0.00207 0.00209 0.00209	0.00208	104	-
	After 10 days in a cold[c)] place and 24 hours at room temperature[d)]	0.00210 0.00209 0.00208	0.00209	-	100
0.02	Initial	0.0203 0.0203 0.0203	0.0203	102	-
	After 10 days in a cold[c)] place and 24 hours at room temperature[d)]	0.0202 0.0204 0.0202	0.0203	-	100

a) Acceptable range: 100±10%
b) Remaining (%, n=3), acceptable limit: ≥90%
c) In the refrigerator, acceptance range of temperature: 5±3°C
d) In the incubator, acceptance range of temperature: 15-30°C
Dates of analysis: May 12, 2014 and May 23, 2014
-: Not calculated

(Excerpted from the submission)

Necropsy

Gross pathology was in the dams was unremarkable. Gross pathology in the placenta was unremarkable.

Cesarean Section Data

Table 74. Pregnancy parameters in rabbits

Dose (mg/kg/3days)	0	0.1	0.3	1.0
No. Dams	16	18	18	19
Corpora lutea	9.9	9.4	10.7	8.8
Implantation sites	8.5	7.7	9.7	7.4
Live fetuses	8.3	7.3	9.3	6.7
Dead fetuses	0.2	0.2	0.1	0.1
Total resorptions	0	0	0	0
Pre-implantation loss	14.1	18.2	8.9	14.2
Post-implantation loss	2.7	5.8	4.6	8.6
Mean fetus weight (g)	32.6/33.2	34.7/35.6	32.4/32.8	32.8/34.6

Mean fetus weight (g) given in female/male

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Offspring

Table 75. Fetal malformations, abnormalities, and variations in rabbits

		Litter			
Dose (mg/kg)		0	0.1	0.3	1.0
Major Malformations (frequencies %)					
Numbers Examined -*External*		132	131	168	128
Tail	shortened	-	-	-	1.8
Minor Visceral Abnormalities					
Numbers Examined		132	131	168	128
Eye	Micropthalmia	-	-	0.9	-
Lung	Absent pulmonary intermedial lobe	3.6	3.6	0.9	4
Kidney	Hemorrhagic kidney	-	1.1	-	-
	Small kidney	-	1.1	-	-
	Malpositioned kidney	-	1.1	-	-
Minor Visceral Variations					
Lung	Short lung fissure	-	-	-	1.2
	Small pulmonary intermedial lobe	1.9	-	1.5	0.5
Liver	Hyperfissured	-	-	0.6	-
Minor Skeletal Abnormalities					
Numbers Examined		132	131	168	128
Thoracic arch	Supernumerary site	-	-	-	0.6
Hyoid	Misshapen hyoid	-	1.2	-	-
Vertebral column	Absent lumbar vertebrae	-	0.5	-	-
	Segmentation defect of lumbar vertebrae	-	-	0.5	-
	Unossified caudal vertebra	-	-	-	0.7
	Fused caudal vertebra	-	-	-	3.3
Minor Skeletal Variations					
Ribs	Cervical rib	0.7	0.7	1.3	2.1
	Full supernumerary rib	54.6	48	75	83.7
Vertebral column	Asymmetric sternebra	-	-	0.6	-
	Fused sternebra (Peduncular)	-	0.5	0.5	-
	Variation in the number of lumbar vertebrae	21.9	13.8	33.7	50.1

(-): no change/no toxicologically significant change

9.3 Prenatal and Postnatal Development

Studies not conducted.

10 Special Toxicology Studies

Studies not conducted.

11 Integrated Summary and Safety Evaluation

The nonclinical studies submitted to this NDA provide sufficient information to support the use of Ninlaro (ixazomib) for the treatment of patients with multiple myeloma who have received at least one prior therapy.

Ixazomib is a second-generation proteasome inhibitor that reversibly binds and inhibits the chymotrypsin-like activity of the β5 subunit of the 20S proteasome. In vitro, ixazomib induces caspase mediated apoptosis in multiple myeloma cell lines. In multiple myeloma cell lines, ixazomib also had a synergistic effect on cytotoxicity in combination with lenalidomide. Ixazomib was cytotoxic in culture to primary cells harvested from multiple myeloma patients who had relapsed after prior therapies. In vivo pharmacology studies using a human myeloma xenograft SCID mouse model following treatment with ixazomib confirmed dose-dependent proteasome inhibition by modulation of β5 subunit activity, other pharmacodynamics markers of cell death and also showed reduced tumor volumes.

Stand-alone safety pharmacology studies were not conducted for the respiratory or nervous system. In the reviewed GLP-compliant repeat-dose toxicology studies: ixazomib exposure did not result in obvious clinical signs of toxicity; no adverse effects were observed on the respiratory systems of rats or dogs; and there were no acute nervous system-related effects, as assessed in part through incorporation of a functional observational battery in the 9 month toxicology study in dogs. There were chronic toxicities in the nervous system in dogs and these findings are outlined in the general toxicology section. When ixazomib was given orally to dogs in a cardiovascular telemetry study, no adverse effects were observed on any cardiovascular parameters.

Based on the data collected in general toxicology studies, there were no gender differences in exposure to oral ixazomib, and increases in C_{max} and AUC values were dose proportional. Intravenous administration of 2 mg/kg ixazomib and oral administration of 6 mg/kg ixazomib resulted in similar plasma area under the concentration-time curves from the time 0 to 24 hours ($AUC_{0\text{-}24hr}$). Tissue distribution was extensive following administration of oral ixazomib. The highest exposure (by C_{max} or AUC) was in the urinary bladder, lymph node, spleen, adrenal glands, kidney (renal cortex and medulla), bile, GI tract, and liver. The longest observed half-life was for the urinary bladder with a $t_{1/2}$ of up to 946 hours. Upon [redacted] (b) (4), ixazomib citrate ester (MLN9708) is metabolized to the active boronic acid form of the drug, (MLN2238). The elimination half-life after oral dosing in nonclinical species ranged from 30 to 72 hours.

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The general toxicology studies were conducted in the rat and dog via oral (gavage). Nonclinical findings in the rat and dog show that ixazomib targets the gastrointestinal tract, hematopoietic and lymphatic systems, nervous system (dog only). Mortalities in the rat toxicology study were attributable to gastrointestinal toxicity and lymphoid depletion. Toxicities in the gastrointestinal system included epithelial hyperplasia, neutrophilic infiltrates (stomach, large and small intestines, peyers patch, mesenteric lymph nodes), single-cell necrosis in the lamina propria of the small and large intestines, and erosion in the stomach. Toxicities in the hematopoietic and lymphatic systems included lymphoid necrosis and depletion in lymph nodes and spleen correlating with increased absolute counts for total white blood cells, neutrophils, monocytes, and elevated lymphocytes in the rats only. At doses below those associated with a dose limiting toxicity, white blood cell parameters were altered consistent with what is observed during an inflammatory response. Toxicities in the nervous system were only observed in dogs and included neuronal degeneration of the sympathetic, dorsal root, peripheral autonomic, and end organ ganglia, and nerve fiber degeneration of the peripheral nerves and ascending tracts in the dorsal columns of the spinal cord. Changes present at recovery in high dose animals included nerve fiber degeneration of the dorsal root ganglia and an increase in axonal degeneration in the dorsal columns of the spinal cord.

Safety concerns stem from the steep dose-toxicity relationship with ixazomib in the chronic rat and dog toxicology studies. There was a less than 1.5-fold separation in systemic exposure between the limit dose and a dose associated with mortality due to drug-induced injury of the liver and gastrointestinal tract in the rat. In dogs, there was an approximately 2-fold separation in exposure between the NOAEL and doses associated with extensive neuronal pathology in the nervous system.

Ixazomib was not mutagenic in the Ames assay nor was it clastogenic in the bone marrow micronucleus assay in mice. It was negative for DNA damage in Comet study. However it showed clastogenic activity (structural chromosomal aberrations) in the in vitro chromosomal aberration assay in human peripheral blood lymphocytes in the presence or absence of an exogenous metabolic activation system.

Ixazomib was teratogenic in the rat and rabbit. In pregnant rat (0.6 mg/kg; 3.6 mg/m^2) dose range-finding studies, there were decreases in fetal weights, decreased fetal viability, and increased post-implantation losses (at 2.5 times the human AUC when given 4 mg orally, in the rat respectively). In pregnant rabbit (1.0 mg/kg; 12 mg/m^2) dose range-finding studies, there were decreases in fetal weights, decreased fetal viability, and increased post-implantation losses (at 3.2 times the human AUC when given 4 mg orally, in the rabbit). In the definitive rabbit study, increases in fetal skeletal variations/abnormalities (caudal vertebrae, number of lumbar vertebrae, full supernumerary ribs, asymmetric sternebra, shortened tail) were observed at doses greater than or equal to 0.3 mg/kg (3.6 mg/m^2), which were also associated with maternal toxicity. The dose of 0.3 mg/kg (3.6 mg/m^2) in rabbits is approximately 1.9 times the human AUC when given 4mg orally. Thus, administration of ixazomib during pregnancy may pose a risk to the human fetus.

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12 Appendix/Attachments

None

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

EMILY J PLACE
11/09/2015

CHRISTOPHER M SHETH
11/09/2015