2.6.7.6 反復投与毒性試験：重要な試験以外の試験

2.6.7.6 Repeat-Dose Toxicity: Non-Pivotal Studies

Test Article: Ixazomib

Species/ Strain	Method of Administration (Vehicle/Formulation)	Duration of Dosing	Dose (mg/kg)	Number and Sex per Group	NOEL (mg/kg)	Report
Rat/ Sprague-Dawley	PO (10% HP-β-CD/2.8% mannitol)	11 days (1 cycle) (Days 1, 4, 8, and 11)	0.3, 0.6, and 1.0	Males, 5/group for main study + 3/group for TK	0.3	4.2.3.2-1

Noteworthy findings:

Mortality: None.

Clinical signs: At 1.0 mg/kg: decreased defecation, soft stools, body surface staining.

BW: At 0.6 and 1.0 mg/kg: decreased BW on Day 7.

Hematology:

At 0.6 mg/kg: On Day 12: increased absolute NEUT.

At 1.0 mg/kg: On Day 12: increased WBC count and absolute NEUT, decreased PLT.

Serum Chemistry:

At 0.6 mg/kg: On Day 12: increased ALT, AST, SDH, and glucose.

At 1.0 mg/kg: On Day 12: increased glucose.

Organ weights:

At 0.6 and 1.0 mg/kg: decreased thymus weight.

At 1.0 mg/kg: decreased spleen weight, increased liver weight.

Macroscopic examination: At 0.6 and 1.0 mg/kg: small thymus.

Microscopic examination:

At 0.6 and 1.0 mg/kg: small and large intestine mucosal hyperplasia and neutrophilic infiltrates; thymus cortical lymphoid depletion; spleen sinusoidal neutrophilic leukocytosis; mesenteric lymph node lymphoid necrosis and neutrophilic infiltrates; and bone marrow hypocellularity.

At 1.0 mg/kg: luminal distension of the stomach and small intestine.

Day 11 plasma TK:

At 0.3 mg/kg: C_{max} 9.38 ng/mL, AUC_{24} 204 ng·hr/mL.

At 0.6 mg/kg: C_{max} 25.9 ng/mL, AUC_{24} 480 ng·hr/mL.

At 1.0 mg/kg: C_{max} 37.9 ng/mL, AUC_{24} 643 ng·hr/mL.

ALT = alanine aminotransferase; AST = aspartate aminotransferase; AUC_{24} = area under the plasma concentration-time curve from the time 0 to 24 hours; BW = body weight; C_{max} = maximum observed plasma concentration; HP-β-CD = hydroxypropyl-beta-cyclodextrin; NEUT = neutrophils; NOEL = no observed effect level; PLT = platelets; PO = oral(ly); SDH = sorbitol dehydrogenase; TK = toxicokinetic(s); WBC = white blood cell.

2.6.7.6 Repeat-Dose Toxicity: Non-Pivotal Studies (continued)

Test Article: Ixazomib

Species/Strain	Method of Administration (Vehicle/Formulation)	Duration of Dosing	Dose (mg/kg)	Number and Sex per Group	NOAEL (mg/kg)	Report
Rat/ Sprague-Dawley	IV (10% HP-β-CD/2.8% mannitol)	11 days (1 cycle) (Days 1, 4, 8, and 11)	0, 0.1, 0.3, and 0.6	Males; 5/group for main study + 3/group for TK	0.1	4.2.3.2-6

Noteworthy findings:

Mortality: At 0.6 mg/kg: All rats on Days 1 - 2.

Clinical signs: At 0.6 mg/kg: hunched posture, decreased activity, lethargy, cold to touch, and soft stools.

BW: At 0.3 mg/kg: decreased BW gain.

Hematology: At 0.3 mg/kg: decreased NEUT, PLT, and RETIC on Day 12.

Serum chemistry: At 0.3 mg/kg: increased GGT on Day 12.

Urinalysis: At 0.3 mg/kg: decreased phosphorus and chloride.

Organ weights: At 0.3 mg/kg: decreased thymus and increased liver weights.

Macroscopic examination: At 0.3 mg/kg: small thymus.

Microscopic examination:

At 0.1 mg/kg: thymus cortical lymphoid single-cell necrosis, colon epithelial single-cell necrosis, and seminal vesicle epithelial single-cell necrosis.

At 0.3 mg/kg: bone marrow hypocellularity; thymus cortical lymphoid depletion and single-cell necrosis; intestinal mucosal hyperplasia/neutrophilic infiltrates/goblet cell loss/epithelial single-cell necrosis; DRG neuronal chromatolysis; hepatocellular single-cell necrosis and cytosegrosomes; epididymis epithelial vacuolization, single-cell necrosis, and neutrophilic infiltrates; seminal vesicle epithelial single-cell necrosis; and stomach neutrophilic infiltrates.

Day 11 plasma TK:

At 0.1 mg/kg: C_{max} 61.8 ng/mL, AUC_{24} 198 ng·hr/mL.

At 0.3 mg/kg: C_{max} 232 ng/mL, AUC_{24} 455 ng·hr/mL.

At 0.6 mg/kg: N/A due to early mortality.

AUC_{24} = area under the plasma concentration-time curve from the time 0 to 24 hours; BW = body weight; C_{max} = maximum observed plasma concentration; DRG = dorsal root ganglia; GGT = gamma glutamyl transferase; HP-β-CD = hydroxypropyl-beta-cyclodextrin; IV = intravenous(ly); N/A = not applicable; NEUT = neutrophils; NOAEL = no observed adverse effect level; PLT = platelets; RETIC = reticulocytes; TK = toxicokinetic(s).

2.6.7.6 Repeat-Dose Toxicity: Non-Pivotal Studies (continued)

Test Article: Ixazomib

Species/Strain	Method of Administration (Vehicle / Formulation)	Duration of Dosing	Dose (mg/kg)	Number and Sex per Group	NOEL (mg/kg)	Report
Dog/beagle	PO (10% HP-β-CD/2.8% mannitol)	QD × 7 days (1 cycle)	0, 0.05, 0.10, and 0.20	Males; 3/group	0.10	4.2.3.2-10

Noteworthy findings:

Mortality: At 0.20 mg/kg: 1 dog on Day 6.

Clinical observations: At 0.20 mg/kg:[a] vomiting, mucoid feces.

Neurological observations: At 0.20 mg/kg:a ataxia, difficulty repositioning limbs, low arousal, and posture of lying on side.

BW: At 0.20 mg/kg:[a] decreased 0.5 kg between Days 1 and 6.

Hematology and serum chemistry: Numerous alterations only observed in the animal euthanized on Day 6; the majority were associated with dehydration, inflammation, or moribundity.

Coagulation: At 0.20 mg/kg: increased FIB in 1 animal surviving until the end of the study.

Macroscopic examination: At 0.20 mg/kg:[a] stomach and intestinal multiple foci of mucosal congestion and/ or hemorrhage.

Microscopic examination: At 0.20 mg/kg: intestinal marked lymphoid necrosis in Peyer's patches[a] and submucosal lymphoid nodules; mesenteric lymph node[a] marked germinal center lymphoid necrosis, follicular and paracortical lymphoid depletion, and moderate capsular neutrophilic infiltrates; sympathetic ganglia (abdominal and thoracic), colonic myenteric plexus [a], and DRG minimal to moderate chromatolysis and cytoplasmic accumulations of eosinophilic granular material; thymic[a] moderate to marked cortical lymphoid necrosis and depletion; small intestine lamina propria minimal to mild lymphoid necrosis and superficial crypt abscess; cecal minimal to mild glandular abscess, mucosal congestion, and hemorrhage; altered hematopoiesis,[a] liver[a] minimal cholestasis and hepatic necrosis; renal[a] minimal tubular epithelial single-cell necrosis; adrenal[a] minimal cortical neutrophilic infiltrates and single-cell necrosis; parathyroid[a] mild chief cell atrophy; prostate[a] mild glandular epithelial single-cell necrosis.

Day 1 plasma TK:	Day 1 blood PD:
At 0.05 mg/kg:[b] C_{max} 11.1 ng/mL, AUC_{24} 118 ng·hr/mL.	At 0.05 mg/kg: E_{max} 23.7 %, AUE_{24} 265 I·hr%
At 0.10 mg/kg: C_{max} 51.2 ng/mL, AUC_{24} 193 ng·hr/mL.	At 0.10 mg/kg: E_{max} 24.4 %, AUE_{24} 403 I·hr%
At 0.20 mg/kg: C_{max} 48.4 ng/mL, AUC_{24} 283 ng·hr/mL.	At 0.20 mg/kg: E_{max} 27.6 %, AUE_{24} 215 I·hr%
Day 7 plasma TK:	Day 7 blood PD
At 0.05 mg/kg: C_{max} 37.5 ng/mL, AUC_{24} 357 ng·hr/mL.	At 0.05 mg/kg: E_{max} 26.3 %, AUE_{24} 482 I·hr%
At 0.10 mg/kg: C_{max} 71.8 ng/mL, AUC_{24} 613 ng·hr/mL.	At 0.10 mg/kg: E_{max} 38.1 %, AUE_{24} 740 I·hr%
At 0.20 mg/kg:[c] C_{max} 104 ng/mL, AUC_{24} 787 ng·hr/mL.	At 0.20 mg/kg: E_{max} 53.6 %, AUE_{24} 996 I·hr%

AUC_{24} = area under the plasma concentration-time curve from the time 0 to 24 hours; BW = body weight; C_{max} = maximum observed plasma concentration; DRG = dorsal root ganglia; FIB = fibrinogen; HP-β-CD = hydroxypropyl-beta-cyclodextrin; NOEL = no observed effect level; PD = pharmacodynamic(s); PO = oral(ly); QD = once daily; TK = toxicokinetic(s).

a Findings were only observed in the animal that was euthanized on Day 6.

b n = 2 (1 animal had no exposure through all time points and was excluded from mean calculation).

c n = 2 (1 animal died before Day 7).

2.6.7.6 Repeat-Dose Toxicity: Non-Pivotal Studies (continued)

Test Article: Ixazomib

Species/Strain	Method of Administration (Vehicle / Formulation)	Duration of Dosing	Dose (mg/kg)	Number and Sex per Group	NOAEL (mg/kg)	Report
Dog/beagle	5-minute IV infusion (10% HP-β-CD/2.8% mannitol)	11 days (1 cycle) (Days 1, 4, 8, and 11)	0, 0.05, 0.10, and 0.20	Males; 3/group	0.10 MTD: 0.20	4.2.3.2-16

Noteworthy findings:

Clinical observations: At all doses: soft feces.

Macroscopic examination: At all doses: dark, SC discoloration at administration site.

Microscopic examination:

At 0.10 mg/kg: mandibular lymph node lymphoid single-cell necrosis.

At 0.20 mg/kg: mesenteric and mandibular lymph node lymphoid single-cell necrosis; DRG neuronal degeneration (chromatolysis and cytoplasmic eosinophilic accumulations); spinal cord dorsal funiculus axonal degeneration and axonophagia; and sympathetic ganglia neuronal degeneration (chromatolysis).

Day 11 plasma TK:

At 0.05 mg/kg: C_{max} 155 ng/mL, AUC_{24} 363 ng·hr/mL.

At 0.10 mg/kg: C_{max} 241 ng/mL, AUC_{24} 552 ng·hr/mL.

At 0.20 mg/kg: C_{max} 742 ng/mL, AUC_{24} 1140 ng·hr/mL .

AUC_{24} = area under the plasma concentration-time curve from the time 0 to 24 hours; C_{max} = maximum observed plasma concentration; DRG = dorsal root ganglia; HP-β-CD = hydroxypropyl-beta-cyclodextrin; IV = intravenous(ly); MTD = maximum tolerated dose; NOAEL = no observed adverse effect level; SC = subcutaneous(ly); TK = toxicokinetic(s).

2.6.7.7 反復投与毒性試験：重要な試験

2.6.7.7A Repeat-Dose Toxicity: A Repeat-Dose Oral Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague Dawley Rats

Species/strain:	Rat/ Sprague-Dawley	Duration of dosing:	32 days (2 cycles of BIW dosing for 2 weeks, separated by a 10-day nondosing period)	Test article:	Ixazomib
Initial age:	7 - 10 weeks	Duration of recovery period:	14 days	Report:	4.2.3.2-2
Date of first dose:	[redacted]	Day of first dose (Day 0 or Day 1):	Day 1	GLP compliance:	Yes
Method of administration:	Oral gavage				
Vehicle/formulation:	[redacted] 1% (w:w) propylene glycol, [redacted]				
Special features:	Pharmacodynamic analysis (20S proteasome activity, assessed by fluorometry) was done in a separate, non-GLP-compliant study (Report 4.2.3.2-3).				
Study endpoint:	STD10 = 1.0 mg/kg				

A Repeat-Dose Oral Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats

Daily dose (mg/kg)	0 (Control)		0.4		0.8		1.2/1.0[a]	1.0[a]
Number of animals (toxicokinetics)[b]	M:3	F:3	M:9	F:9	M:9	F:9	M:9	F:9
Toxicokinetics								
C_{max} (ng/mL)								
Day 1	N/A		25.6		73.0		147	123
Day 11	N/A		23.3		40.2		50.1	N/A
Day 22	N/A		12.2		30.0		36.0	N/A
Day 32	N/A		13.1		29.6		41.2	N/A
AUC_{24} (ng·hr/mL)								
Day 1	N/A		302		591		711	819
Day 11	N/A		315		438		577	N/A
Day 22	N/A		209		288		311	N/A
Day 32	N/A		244		373		521	N/A

2.6.7.7A Repeat-Dose Toxicity: A Repeat-Dose Oral Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague Dawley Rats (continued)

A Repeat-Dose Oral Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats										
Daily dose (mg/kg)	0 (Control)		0.4		0.8		1.2/1.0[a]		1.0[a]	
Number of animals (pharmacodynamic)[b]	M:3	F:3	M:9	F:9	M:9	F:9	M:9		F:9	
Pharmacodynamics										
E_{max} (I%)										
Day 1	N/A	N/A	23.5	54.9	36.4	71.4	54.3		63.2	
Day 11	N/A	N/A	38.2	48.4	33.5	74.7	41.3		N/A	
Day 22	N/A	N/A	51.5	61.5	60.5	54.1	60.6		N/A	
Day 32	N/A	N/A	64.5	33.3	61.1	59.4	59.0		N/A	
AUE_{24} (hr·I%)										
Day 1	N/A	N/A	484	934	596	1190	740		947	
Day 11	N/A	N/A	471	950	631	1160	720		N/A	
Day 22	N/A	N/A	1150	358	1190	570	1180		N/A	
Day 32	N/A	N/A	744	528	819	820	824		N/A	
Noteworthy findings										
Number of Animals (main and recovery)	M:15	F:15	M:15	F:15	M:15	F:15	M:15		F:15	
Died or euthanized moribund	1[c]	1[c]	1[c]	1[c]	3[c]	2[c]	6[c, d]		5[d]	
Body weight (g)[e]										
Day 32	433.10	275.72	–	–	–13**	–	–17**		–	
Food consumption (g/day)[e]										
Days 29 – 32	35.2	26.7	–	–	–	–	25.9**		–	
Number of Animals (main and recovery)	M:15	F:15	M:15	F:15	M:15	F:15	M:15		F:15	
Clinical observations[f]							M: 6 (ED)	M: 4 (MS)	F: 5 (ED)	F: 10 (MS)
Cold to touch	–	–	–	–	–	–	+	–	+	–
Hunched posture	–	–	–	–	–	–	+	–	+	–
Labored breathing	–	–	–	–	–	–	–	–	+	–
Lethargy	–	–	–	–	–	–	+	–	+	–
Mucoid or soft feces	–	–	–	–	–	–	+	+	+	+
Porphyrin staining	–	–	–	–	–	–	+	+	+	+
Rough hair coat							–	+	–	–
Urine stain	–	–	–	–	–	–	+	+	+	+

2.6.7.7A Repeat-Dose Toxicity: A Repeat-Dose Oral Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague Dawley Rats (continued)

A Repeat-Dose Oral Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats										
Daily dose (mg/kg)	0 (Control)		0.4		0.8		1.2/1.0[a]		1.0[a]	
Number of Animals (main and recovery)	M:15	F:15	M:15	F:15	M:15	F:15	M:15		F:15	
Hematology and coagulation[e]										
Day 32										
NEUT, abs (10^3/μL)	1.164	0.816	+138**	+167**	+771**	+474**	+852**		–	
BASO, abs (10^3/μL)	0.024	–	–	–	+167**	–	+167**		–	
EOS, abs (10^3/μL)	0.080	–	–	–	–	–	–51**		–	
HCT (%)	45.25	41.41	–6*	–	–7*	–6*	–6*		–	
HGB (g/dL)	15.57	14.69	–6*	–	–6*	–8*	–		–	
LUC, abs (10^3/μL)	0.096	0.046	–	–	+372**	+122**	+202**		–	
MCH (pg)	19.47	–	–4*	–	–6*	–	–4*		–	
MCV (fL)	56.64	–	–4*	–	–6*	–	–5*		–	
MONO, abs (10^3/μL)	0.139	0.089	–	–	+201**	+122**	+240**		–	
PLT (10^3/μL)	1121.1	1191.6	–	–23*	–26**	–31*	–28**		–	
RETIC, abs (10^9/L)	202.65	–	–	–	–29**	–	–24		–	
WBC (10^3/μL)	8.91	6.60	–	+49**	+127*	+89**	+135*		–	
FIB (mg/dL)	244.3	205.0	–	–	+40**	+32**	+21**		–	
Clinical chemistry[e]										
Day 32										
ALP (U/L)	144.8	81.4	–34**	–41**	–22	–46**	–30**		–	
ALT (U/L)	–	30.7	–	–21*	–	–39*	–		–	
AST (U/L)	–	106.3	–	–	–	–32**	–		–	
BUN (mg/dL)	15.6	16.0	–14**	–18*	–	–23*	–12**		–	
CHOL (mg/dL)	71.7	–	–	–	–32*	–	–33*		–	
Primary necropsy findings (Day 33)										
Number of animals (main study and early death)	M:9	F:9	M:9	F:9	M:7	F:8	M:5 (ED)	M:4 (MS)	F:5 (ED)	F:10 (MS)
Organ weights (g) (absolute)[e, g]										
Liver	–	7.12	–	+25**	–	+29**	–	–	N/A	
Thymus	0.4684	0.4134	–	–	–48*	–35*	–	–35	N/A	

2.6.7.7A Repeat-Dose Toxicity: A Repeat-Dose Oral Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague Dawley Rats (continued)

A Repeat-Dose Oral Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats										
Daily dose (mg/kg)	0 (Control)		0.4		0.8		1.2/1.0[a]		1.0[a]	
Number of animals (main study and early death)	M:9	F:9	M:9	F:9	M:7	F:8	M:5 (ED)	M:4 (MS)	F:5 (ED)	F:10 (MS)
Organ weights (g) (relative to body weight)[e, g]										
Adrenal gland	0.0143	–	–	–	+49	–	–	+38**	N/A	
Liver	2.89	2.82	–	+19**	+10**	+31**	–	+19**	N/A	
Thymus	0.1156	0.1611	–	–	–41*	–31	–	–24	N/A	
Macroscopic findings[h]										
Stomach										
Distended	–	–	2	–	2	–	–	4	–	–
Thymus										
Small	–	–	–	–	2	–	–	–	–	–
Microscopic findings[h]										
Adrenal gland										
Vacuolation, zona fasciculata	0	1	3	1	7	4	2	3	4	2
Minimal	–	1	3	1	6	4	1	3	–	2
Mild	–	–	–	–	1	–	1	–	2	–
Moderate	–	–	–	–	–	–	–	–	2	–
Necrosis, zona fasciculata	–	–	–	–	–	–	2	–	2	–
Minimal	–	–	–	–	–	–	2	–	–	–
Mild	–	–	–	–	–	–	–	–	1	–
Moderate	–	–	–	–	–	–	–	–	1	–
Necrosis, zona glomeruta	–	–	–	–	–	–	1	–	–	–
Moderate	–	–	–	–	–	–	1	–	–	–
Bone Marrow[i]										
Cellular depletion	0	0	0	0	0	0	5	0	5	0
Minimal	–	–	–	–	–	–	–	–	3	–
Mild	–	–	–	–	–	–	2	–	2	–
Moderate	–	–	–	–	–	–	3	–	–	–
Intestine[j]										
Inflammation, acute, mucosa	0	0	2	1	6	0	5	4	4	1
Minimal	–	–	1	1	1	–	1	1	1	1
Mild	–	–	1	–	5	–	4	3	2	–

A Repeat-Dose Oral Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats										
Daily dose (mg/kg)	0 (Control)		0.4		0.8		1.2/1.0[a]		1.0[a]	
Moderate	–	–	–	–	–	–	–	–	1	–

2.6.7.7A Repeat-Dose Toxicity: A Repeat-Dose Oral Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague Dawley Rats (continued)

A Repeat-Dose Oral Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats										
Daily dose (mg/kg)	0 (Control)		0.4		0.8		1.2/1.0[a]		1.0[a]	
Number of animals (primary necropsy and early death)	M:9	F:9	M:9	F:9	M:7	F:8	M:5 (ED)	M:4 (MS)	F:5 (ED)	F:10 (MS)
Intestine[j]										
Inflammation, acute, submucosal	0	0	0	0	3	0	1	0	4	2
Minimal	–	–	–	–	–	–	–	–	1	–
Mild	–	–	–	–	3	–	1	–	–	–
Moderate	–	–	–	–	–	–	–	–	1	–
Mucosal thickening	0	0	2	5	6	7	2	2	0	0
Minimal	–	–	2	4	5	5	1	1	–	–
Mild	–	–	–	1	1	2	1	1	–	–
Lymph node[k]										
Depletion, lymphoid	0	0	0	0	2	2	2	0	3	0
Minimal	–	–	–	–	1	2	1	–	1	–
Mild	–	–	–	–	–	–	1	–	2	–
Moderate	–	–	–	–	1	–	–	–	–	–
Peyer's patch										
Depletion, lymphoid	0	0	0	0	2	1	5	0	3	0
Minimal	–	–	–	–	1	1	1	–	–	–
Mild	–	–	–	–	1	–	3	0	3	0
Moderate	–	–	–	–	–	–	1	–	–	–
Spleen										
Depletion, lymphoid	0	0	0	0	1	0	5	0	5	0
Minimal	–	–	–	–	1	–	1	–	2	–
Mild	–	–	–	–	–	–	4	–	3	–
Thymus										
Depletion, cellular, cortex	0	0	0	0	3	2	1	0	0	0
Minimal	–	–	–	–	1	–	–	–	–	–
Mild	–	–	–	–	–	2	1	–	–	–
Moderate	–	–	–	–	1	–	–	–	–	–
Marked	–	–	–	–	1	–	–	–	–	–

2.6.7.7A Repeat-Dose Toxicity: A Repeat-Dose Oral Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague Dawley Rats (continued)

A Repeat-Dose Oral Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats										
Daily dose (mg/kg)	0 (Control)		0.4		0.8		1.2/1.0[a]		1.0[a]	
Number of animals (primary necropsy and early death)	M:9	F:9	M:9	F:9	M:7	F:8	M:5 (ED)	M:4 (MS)	F:5 (ED)	F:10 (MS)
Necrosis, cortex	0	0	0	0	0	0	0	0	4	0
Minimal	–	–	–	–	–	–	–	–	1	–
Mild	–	–	–	–	–	–	–	–	2	–
Moderate	–	–	–	–	–	–	–	–	1	–
Bone marrow smear analysis[l]										
M:E ratio	1.1	–	–	–	2.5	–	1.4		–	
Total myeloid cell count	162.4	–	–	–	215.0	–	226.0		–	
Myeloblast/promyelocyte	6.0	–			14.0	–	17.8		–	
Myelocyte	12.1	–	–	–	–	–	23.5		–	
Metamyelocyte	7.1	–	–	–	8.3	–	17.8		–	
Band neutrophil	22.9	–			31.1	–	39.5		–	
Segmented neutrophil	101.0	–	–	–	140.1	–	120.3		–	
Miscellaneous cells										
Lymphocyte	176.4	190.6	–	–	128.1	136.6	91.0		–	
Recovery evaluations										
Number of Animals (recovery)	M:5	F:5	M:5	F:5	M:5	F:5	M:5		F:0	
Clinical observations[f]										
Rough hair coat	–	–	–	–	–	–	1		–	
Body weight (g)[e, m]	484.08	306.86	–9	–9	–7	–11*	–12*		–	
Hematology[e,n]										
PLT ($10^3/\mu L$)	1107.0	–	–	–	+30**	–	+35		N/A	
RETIC, abs ($10^9/L$)	181.94	–	–	–	–	–	+86		N/A	

* $p \leq 0.05$ (Dunnett test); ** $p \leq 0.05$ (Kruskal-Wallis with Dunn procedure); + = findings observed; – = no noteworthy findings; abs = absolute; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; AUE_{24} = area under the plasma concentration-time curve from the time 0 to 24 hours; AUE_{24} = area under the effect-time curve from time 0 to 24 hours; BASO = basophil; BIW = twice weekly; BUN = blood urea nitrogen; CHOL = cholesterol;

Cmax = maximum observed plasma concentration; ED = early death; Emax = maximum effect; EOS = eosinophil; F = female(s), FIB = fibrinogen; GLP = Good Laboratory Practice; HCT = hematocrit; HGB = hemoglobin; I% = percentage of proteasome inhibition; LUC = large unstained cell; M = male(s); MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; M:E = myeloid-to-erythroid ratio; MONO = monocyte; MS = main study; MTD = maximum tolerated dose; N/A = not applicable; NEUT = neutrophil; PLT = platelet; RETIC = reticulocyte; STD10 = severely toxic dose in 10% of animals administered test article; WBC = white blood cell; w:w = weight-to-weight ratio.

a Due to severe toxicity, Group 4 males received 1.2 mg/kg on Day 1 only. The dose in Group 4 males was reduced to 1.0 mg/kg for subsequent administrations. Due to severe toxicity, Group 4 females received 1.0 mg/kg on Day 1 only. Dosing was discontinued for all Group 4 females after Day 1.

b For animals receiving text article, n = 9/sex/dose group, but 3/sex/dose group/time point. Due to lack of pronounced sex-related differences in exposure to ixazomib, data is presented as sexes combined.

c The deaths in rats at 0, 0.4, and 0.8 mg/kg were considered to be procedure related and not due to administration of ixazomib. The relationship between test article administration and death in the rat at 1.2/1.0 mg/kg was undetermined as this rat was inadvertently placed in the freezer, and therefore, pathology examination was not performed on this animal.

d Test article-related mortality occurred in 5 males and 5 females from Group 4 on Days 2 to 3. The cause of death/moribund condition necessitating humane sacrifice on Days 2 to 3 after a single dose of ixazomib in Group 4 males and female rats was metabolic stress, likely precipitated by intestinal mucosal toxicity and associated fluid and electrolyte imbalances. Bone marrow cellular depletion was also seen in these rats.

e For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

f +/– indicates presence or absence of finding.

g Both absolute and relative weights differed from controls in the direction indicated. Number indicates percent difference for the absolute organ weights.

h The numbers represent the total number of incidences in tissues examined.

i Includes findings from sternum and femur. When severity of findings differed amongst sites, the highest level of severity is presented.

j Includes all sections of the intestine. When severity of findings differed amongst sites, the highest level of severity is presented.

k Includes both mandibular and mesenteric lymph nodes. When severity of findings differed amongst sites, the highest level of severity is presented.

l The number represents the group mean. Bone marrow smears not taken from early death animals.

m At end of the recovery period (Day 46).

n At end of the recovery period (Day 47).

2.6.7.7B Repeat-Dose Toxicity: A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period

Species/strain:	Rat/Sprague-Dawley	Duration of dosing:	94 days (5 cycles of BIW dosing for 2 consecutive weeks separated by a 10-day nondosing observation period)	Test article:	Ixazomib citrate
Initial age:	7 - 8 weeks	Duration of recovery period:	14 days	Report:	4.2.3.2-4
Date of first dose:	[redacted]	Day of first dose (Day 0 or Day 1):	Day 0	GLP compliance:	Yes
Method of administration:	Oral gavage				
Vehicle/formulation:	[redacted] and 0.45% NaCl, in sterile Water For Injection, USP [redacted]				
Special features:	None				
Study endpoint:	MTD = 0.6 mg/kg				

A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period								
Daily dose (mg/kg)[a]	0 (Control)		0.2		0.4		0.8/0.6[b]	
Number of animals (toxicokinetic)[c]	M:6	F:6	M:18	F:18	M:18	F: 18	M:18	F:18
Toxicokinetics								
C_{max}(ng/mL)								
Day 0	N/A		4.19		11.3		29.2	
Day 94	N/A		8.53		18.4		26.1	
AUC_{24} (ng·hr/mL)								
Day 0	N/A		92.7		193		395	
Day 94	N/A		156		253		296	

2.6.7.7B Repeat-Dose Toxicity: A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period (continued)

A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period										
Daily dose (mg/kg)[a]	0 (Control)		0.2		0.4		0.8/0.6[b]			
Number of animals (main and recovery)	M:15	F:15	M:10	F:10	M:15	F:15	M:15		F:15	
Noteworthy findings										
Died or euthanized moribund	1[d]	1[e]	–	–	–	–	1[f]		2[e, f]	
Body weight (g)[g, h]	573	292	–	–	–	–	–11.3**		–2.7	
Food consumption (g/day)[g, h]	29	19	–	–	–13.8*	–10.5*	–24.1**		–5.3	
Clinical observations[i]							M:1 (ED)	M:14 (MS)	F:2 (ED)	F:13 (MS)
Body cool to the touch	–	–	–	–	–	–	+	–	+	–
Decreased defecation	–	–	–	–	–	–	+	–	+	–
Dermal atonia	–	–	–	–	–	–	+	–	+	–
Extremities cool to the touch	–	–	–	–	–	–	+	–	–	+
Feces smaller than normal	–	–	–	–	–	–	+	–	+	+
Hypoactivity	–	–	–	–	–	–	+	–	+	–
Labored respiration	–	–	–	–	–	–	+	–	–	–
Pale extremities	–	–	–	–	–	–	+	–	+	–
Thin appearance	–	–	–	–	–	–	+	–	+	–
Hematology[g]										
BASO, abs (10^3/μL)	0.02	0.01	0.03	0.02	0.05**	0.03	0.05**		0.05**	
EOS, abs (10^3/μL)	0.14	0.10	0.14	0.09	0.09*	0.08	0.07**		0.08	
PLT (10^3/μL)	1262	1229	1199	1128	1066**	912**	973**		902**	
LYMPH, abs (10^3/μL)	9.17	5.10	11.32	8.17	14.04**	10.17**	14.55**		12.99**	
MONO, abs (10^3/μL)	0.23	0.08	0.21	0.10	0.36	0.16	0.47**		0.25**	
NEUT, abs (10^3/μL)	1.65	0.78	2.58	0.99	6.90*	2.92	12.64**		5.52**	
WBC (10^3/μL)	11.30	6.11	14.38	9.43	21.59**	13.46**	27.92**		19.05**	

2.6.7.7B Repeat-Dose Toxicity: A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period (continued)

A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period										
Daily dose (mg/kg)[a]	0 (Control)		0.2		0.4		0.8/0.6[b]			
Number of animals (main and recovery)	M:15	F:15	M:10	F:10	M:15	F:15	M:15		F:15	
Clinical chemistry[g]										
Glucose (mg/dL)	138	122	152	121	145	126	143		140*	
BUN (mg/dL)	14.1	17.9	13.1	15.9	12.2*	13.0**	11.8**		12.7**	
Urinalysis[g]										
Corrected total calcium (mg/kg)	0.58	3.13	0.48	2.82	0.26	1.33**	0.52		1.12**	
Corrected total chloride (mg/kg)	24.52	40.88	24.69	29.62	17.62	21.80**	17.59		21.50**	
Urine protein (> 1+)	+	–	+	–	+	–	+		+	
Primary necropsy findings (Day 94)										
Number of animals (main study and early death)	M:10	F:10	M:10	F:10	M: 10	F:10	M:1 (ED)	M:10 (MS)	F:2 (ED)	F:10 (MS)
Organ weights[h, j]										
Adrenal gland										
Absolute (g)	0.0606	0.0592	+5.3	+14.9	+7.6	+8.8	+5.0		+19.8**	
Relative to FBW (g/100 g)	0.012	0.022	0.0	+18.2*	0.0	+9.1	+8.3		+18.2*	
Liver										
Absolute (g)	15.21	7.69	+11.3	+8.8	+8.4	+8.3	–5.3		+19.1**	
Relative to FBW (g/100 g)	2.874	2.861	+5.8	+11.1**	+8.2	+8.0	+5.5		+18.9**	

2.6.7.7B Repeat-Dose Toxicity: A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period (continued)

A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period										
Daily dose (mg/kg)[a]	0 (Control)		0.2		0.4		0.8/0.6[b]			
Number of animals (main study and early death)	M:10	F:10	M:10	F:10	M:10	F:10	M:1 (ED)	M:10 (MS)	F:2 (ED)	F:10 (MS)
Microscopic findings[k]										
Aorta										
Atrophy, brown adipose tissue	0	0	NE[l]	NE	NE	NE	1	0	1	0
Moderate	–	–	–	–	–	–	1	–	1	–
Intestine[m]										
Degeneration, epithelial	0	0	0	0	0	0	1	0	0	0
Moderate	–	–	–	–	–	–	1	–	–	–
Hyperplasia, epithelial	1	0	3	3	8	10	1	9	2	10
Minimal	1	–	3	3	8	10	–	2	–	4
Mild	–	–	–	–	–	–	1	7	1	6
Moderate	–	–	–	–	–	–	–	–	1	–
Inflammation, acute	0	0	1	2	6	4	1	5	2	4
Minimal	–	–	1	2	6	4	–	5	2	4
Mild	–	–	–	–	–	–	1	–	–	–
Necrosis, single cell	0	0	1	1	3	0	1	1	2	1
Minimal	–	–	1	1	3	–	–	1	2	1
Mild	–	–	–	–	–	–	1	–	–	–

2.6.7.7B Repeat-Dose Toxicity: A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period (continued)

A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period										
Daily dose (mg/kg)[a]	0 (Control)		0.2		0.4		0.8/0.6[b]			
Number of animals (primary necropsy and early death)	M:10	F:10	M:10	F:10	M:10	F:10	M:1 (ED)	M:10 (MS)	F:2 (ED)	F:10 (MS)
Lymph node[n]										
Depletion, lymphoid	0	1	5	7	4	9	1	9	2	4
Minimal	–	1	5	7	4	8	–	5	–	4
Mild	–	–	–	–	–	1	–	3	1	–
Moderate	–	–	–	–	–	–	1	1	1	–
Necrosis, single cell	6	7	4	7	4	8	1	9	2	7
Minimal	6	7	3	5	4	4	–	7	1	4
Mild	–	–	1	2	–	4	1	2	1	3
Marrow[o]										
Hypocellular	0	0	NE[l]	NE	NE	NE	0	0	1	0
Mild	–	–	–	–	–	–	–	–	1	–
Necrosis, single cell	0	0	NE	NE	NE	NE	1	0	0	0
Mild	–	–	–	–	–	–	1	–	–	–
Peyer's patches										
Depletion, lymphoid	0	0	0	0	0	1	1	3	0	2
Minimal	–	–	–	–	–	1	–	3	–	2
Moderate	–	–	–	–	–	–	1	–	–	–
Necrosis, single cell	2	7	6	5	3	3	0	1	0	3
Minimal	2	7	6	5	3	3	–	1	–	3

2.6.7.7B Repeat-Dose Toxicity: A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period (continued)

A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period										
Daily dose (mg/kg)[a]	0 (Control)		0.2		0.4		0.8/0.6[b]			
Number of animals (primary necropsy and early death)	M:10	F:10	M:10	F:10	M:10	F:10	M:1 (ED)	M:10 (MS)	F:2 (ED)	F:10 (MS)
Spleen										
Depletion, lymphoid	0	0	0[p]	NE[l]	NE	NE	1	0	1	0
Mild	–	–	–	–	–	–	–	–	1	–
Moderate	–	–	–	–	–	–	1	–	–	–
Stomach, glandular										
Degeneration, epithelial	0	0	NE	NE	NE	NE	1	0	0	0
Minimal	–	–	–	–	–	–	1	–	–	–
Thymus										
Depletion, lymphoid	2	2	0[q]	NE	0[q]	0[q]	1	2	1	3
Minimal	2	2	–	–	–	–	–	2	–	3
Moderate	–	–	–	–	–	–	1	–	1	–
Necrosis, single cell	2	1	0[q]	NE	0[q]	0[q]	1	1	1	3
Minimal	2	1	–	–	–	–	–	1	–	3
Mild	–	–	–	–	–	–	–	–	1	–
Moderate	–	–	–	–	–	–	1	–	–	–

2.6.7.7B Repeat-Dose Toxicity: A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period (continued)

A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period								
Daily dose (mg/kg)[a]	0 (Control)		0.2		0.4		0.8/0.6[b]	
Recovery evaluations								
Number of animals (recovery)	M:4	F:4	M:0	F:0	M:5	F:5	M:4	F:3
Body weight (g)[h, r]	617	302	N/A	N/A	–8.3	–2.6	–11.5*	–9.3
Food consumption (g/day)[h, r]	31	21	N/A	N/A	–3.2	0.0	0.0	–4.8
Hematology[r]								
BASO, abs (10^3/μL)	0.01	0.00	N/A	N/A	0.01	0.01	0.01	0.01
EOS, abs (10^3/μL)	0.13	0.08	N/A	N/A	0.11	0.07	0.09	0.09
LYMPH, abs (10^3/μL)	8.33	4.97	N/A	N/A	7.75	5.45	7.17	6.07
MONO, abs (10^3/μL)	0.23	0.10	N/A	N/A	0.14	0.11	0.16	0.10
NEUT, abs (10^3/μL)	1.45	0.81	N/A	N/A	1.31	1.41	1.62	0.84
PLT (10^3/μL)	1194	1398	N/A	N/A	1248	972	1284	1319
WBC (10^3/μL)	10.23	6.02	N/A	N/A	9.38	7.09	9.09	7.19
Clinical chemistry[r]								
BUN (mg/dL)	15.5	19.5	N/A	N/A	15.7	16.5	14.7	16.9
Glucose (mg/dL)	142	124	N/A	N/A	141	119	130	115
Urinalysis[r]								
Urine protein (> 1+)	–	–	N/A	N/A	–	–	–	–
Corrected total calcium (mg/kg)	0.41	1.60	N/A	N/A	0.53	1.48	0.18	0.71
Corrected total chloride (mg/kg)	12.56	21.99	N/A	N/A	16.45	16.28	15.70	13.10

2.6.7.7B Repeat-Dose Toxicity: A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period (continued)

A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period								
Daily dose (mg/kg)[a]	0 (Control)		0.2		0.4		0.8/0.6[b]	
Number of animals (recovery)	M: 4	F:4	M:0	F:0	M:5	F:5	M:4	F:3
Recovery Necropsy (Day 109)								
Organ weights[h]								
Adrenal gland								
Absolute (g)	0.0633	0.0793	N/A	N/A	–13.0	–11.7	–15.2	–6.7
Relative to FBW (g/100 g)	0.011	0.028	N/A	N/A	–9.1	–10.7	–9.1	+7.1
Liver								
Absolute (g)	15.46	8.31	N/A	N/A	–3.6	+4.3	–2.5	–5.2
Relative to FBW (g/100 g)	2.654	2.975	N/A	N/A	+4.6	+6.4	+10.4	+5.0
Microscopic findings[k]								
Intestine[l]								
Hyperplasia, epithelial	0	0	N/A	N/A	2	0	1	0
Minimal	–	–	–	–	2	–	1	–
Lymph node[m]								
Depletion, lymphoid	1	3	N/A	N/A	3	1	3	1
Minimal	1	3	–	–	3	1	3	1
Necrosis, single cell	2	3	N/A	N/A	1	2	2	0
Minimal	2	2	–	–	1	2	2	–
Mild	–	1	–	–	–	–	–	–
Peyer's patches								
Depletion, lymphoid	0	0	N/A	N/A	1	0	0	0
Minimal	–	–	–	–	1	–	–	–
Necrosis, single cell	1	3	N/A	N/A	1	2	0	3
Minimal	1	3	–	–	1	2	–	3

*p < 0.05, **p < 0.01 (Dunnett test); + = findings observed; – = no noteworthy findings; abs = absolute; AUC_{24} = area under the plasma concentration-time curve from the time 0 to 24 hours; BASO = basophil; BIW = twice-weekly; BUN = blood urea nitrogen; Cmax = maximum observed plasma concentration; ED = early death; EOS = eosinophil; F = female(s); FBW = final body weight; GLP = Good Laboratory Practice; LYMPH = lymphocyte; M = male(s); MONO = monocyte; MS = main study; MTD = maximum tolerated dose; N/A = not applicable; NE = not evaluated; NEUT = neutrophil; NOAEL = no observed adverse effect level; PLT = platelet; USP = United States Pharmacopeia; WBC = white blood cell.

a Doses are expressed in terms of mg/kg ixazomib.

b Prior to initiation of Cycle 3, the high dose was lowered from 0.8 to 0.6 mg/kg.

c For animals receiving text article, 18/sex/group were dosed, but blood for toxicokinetic (TK) analysis was only collected from 6/sex/group. The remaining animals were kept as replacements if needed. Due to lack of pronounced sex-related differences in exposure to ixazomib, data is presented as sexes combined.

d Death was not test article-related. The animal was found dead on Day 70 and the cause of death was determined to be gavage error.
e Death was not test article-related. The control female and 1 high-dose female were found dead on Day 95 and the cause of death was determined to be blood collection error.
f Death was test article-related. One male and 1 female were euthanized on Days 29 and 31, respectively, following adverse clinical signs. The cause of death was due to gastrointestinal (GI) and lymphoid toxicity which predisposed the rats to possible sepsis.
g At the end of the dosing period (Week 13).
h For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not percent differences).
I +/– indicates presence or absence of finding.
j Organ weights represent tissues taken at the primary necropsy only and do not include early death animals.
k Numbers indicated total incidence observed in tissues examined.
l Microscopic evaluation not performed on nontarget tissues in 0.2 and 0.4 mg/kg groups.
m Includes findings in duodenum, ileum, jejunum, and rectum. When severity of findings differed amongst sections, the highest level of severity is presented.
n Includes findings in axillary, mandibular, mesenteric, and popliteal lymph nodes. When severity of findings differed amongst lymph nodes, the highest level of severity is presented.
o Includes findings in femur and sternum. When severity of findings differed amongst bone marrow sources, the highest level of severity is presented.
p Although this was not considered a target organ in Group 2 because there were no findings in Group 3, the spleen was microscopically examined in 1 animal after being noted as enlarged upon macroscopic evaluation. The only microscopic finding was minimal lymphoid hyperplasia, which was not considered a noteworthy finding.
q The thymi were microscopically examined in 2 males in Group 2, and 2 males and 1 female in Group 3 after being noted as dark red upon macroscopic evaluation.
r At the end of the recovery period (Week 15).

2.6.7.7C Repeat-Dose Toxicity: A 6-Month Oral Gavage Toxicity Study of MLN9708 With a 2-Week Recovery Period in Sprague-Dawley Rats

Species/strain:	Rat/ Sprague-Dawley	Duration of dosing:	182 Days (7 dosing cycles of 3 QW doses followed by a 13-day nondosing period)	Test article:	Ixazomib citrate
Initial age:	7 - 8 weeks	Duration of recovery period:	14 days	Report:	4.2.3.2-5
Date of first dose:	[redacted]	Day of first dose (Day 0 or Day 1):	Day 0	GLP compliance:	Yes
Method of administration:	PO				
Vehicle/formulation:	[redacted] and 0.45% NaCl, in SWI, USP [redacted]				
Special features:	None				
Study endpoint:	NOAEL = 0.2 mg/kg; MTD = 0.8 mg/kg (males); 0.6 mg/kg (females)				

A 7-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period

Daily dose (mg/kg)[a]	0 (Control)		0.2		0.4		0.8/0.6[b]	
Number of animals (toxicokinetic)[c]	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15[d]
Toxicokinetics								
C_{max} (ng/mL)								
Day 0	N/A		3.68	5.12	8.97	13.0	21.4	66.4
Day 168	N/A		5.42	6.55	19.2	21.3	62.1	44.7
AUC_{24} (ng·hr/mL)								
Day 0	N/A		75.4	89.6	149	183	282	496
Day 168	N/A		98.2	116	189	211	252	288
AUC_{168} (ng·hr/mL)								
Day 0	N/A		264	374	655	742	1150	1660
Day 168	N/A		489	476	754	803	1200	1070

2.6.7.7C Repeat-Dose Toxicity: A 6-Month Oral Gavage Toxicity Study of MLN9708 With a 2-Week Recovery Period in Sprague-Dawley Rats (continued)

A 7-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period									
Daily dose (mg/kg)[a]	0 (Control)		0.2		0.4		0.8/0.6[b]		
Noteworthy findings									
Number of animals (main and recovery)	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15	
Died or euthanized moribund	–	–	–	–	2[e]	–	–	5[f]	
Body weight (g)[g, h]	687	365	–	–	–	–	–11.1**	–10.1	
Number of animals (main and recovery)	M:_15	F:_15	M:_15	F:_15	M:_15	F:_15	M:_15	F:_10 (MS)	F:_5 (ED)
Clinical observations[i]									
Body cool to touch	–	–	–	–	–	–	–	–	+
Clear discharge of eyes	–	–	–	–	–	–	–	+	+
Decreased defecation, soft/small feces	–	–	–	–	–	–	+	+	+
Diarrhea	–	–	–	–	–	–	+	–	+
Hypoactivity	–	–	–	–	–	–	+	–	–
Partial closure of eyes	–	–	–	–	–	–	–	–	+
Red material around nose	–	–	–	–	+	–	–	+	+
Thin	–	–	–	–	–	–	+	–	–
Yellow material around urogenital/anogenital/forelimb area	–	–	–	–	–	–	+	+	+

2.6.7.7C Repeat-Dose Toxicity: A 6-Month Oral Gavage Toxicity Study of MLN9708 With a 2-Week Recovery Period in Sprague-Dawley Rats (continued)

A 7-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period									
Daily dose (mg/kg)[a]	0 (Control)		0.2		0.4		0.8/0.6[b]		
Number of animals (main study)	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10	
Hematology and coagulation[g, h]									
WBC ($10^3/\mu L$)	7.78	5.21	–	–	+105.3**	+54.5	+151.3**	+77.2**	
NEUT ($10^3/\mu L$)	1.92	1.01	–	–	+304.7**	+151.5	+530.2**	+248.5*	
LYMPH ($10^3/\mu L$)	5.30	3.88	–	–	+39.2*	+29.9	+23.6	+36.6	
MONO ($10^3/\mu L$)	0.35	0.18	–	–	+62.9*	+72.2*	+97.1**	+44.4	
BASO ($10^3/\mu L$)	0.01	0.01	–	–	+100.0	–	+100.0**	–	
APTT (seconds)	15.0	13.7	–	–	–	–	–	+10.2**	
Number of animals (main study and early death)	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10 (MS)	F:5 (ED)
Primary Necropsy (Day 183)									
Microscopic findings[j, k]									
Adrenal Cortex									
Degeneration/Necrosis, Zona Fasciculata	0	0	0	NE	0	0	0	0	5
Minimal	–	–	–	–	–	–	–	–	2
Mild	–	–	–	–	–	–	–	–	3
Decreased vacuolation	1	0	5	NE	2	0	5	0	0
Minimal	1	–	5	–	2	–	3	–	–
Mild	–	–	–	–	–	–	2	–	–
Adrenal Medulla									
Decreased secretory granules	0	0	NE	NE	1	0	0	0	5
Minimal	–	–	–	–	0	–	–	–	1
Mild	–	–	–	–	1	–	–	–	4
Necrosis, single cell	0	0	NE	NE	0	0	0	0	1
Minimal	–	–	–	–	–	–	–	–	1

2.6.7.7C Repeat-Dose Toxicity: A 6-Month Oral Gavage Toxicity Study of MLN9708 With a 2-Week Recovery Period in Sprague-Dawley Rats (continued)

A 7-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period									
Daily dose (mg/kg)[a]	0 (Control)		0.2		0.4		0.8/0.6[b]		
Number of animals (main study and early death)	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10 (MS)	F:5 (ED)
Intestine[l]									
Atrophy, villous	0	0	0	0	0	0	0	0	2
Mild	–	–	–	–	–	–	–	–	2
Hyperplasia, epithelial	0	0	10	7	10	10	10	10	4
Minimal	–	–	5	6	3	3	1	2	1
Mild	–	–	5	1	7	6	9	5	3
Moderate	–	–	0	0	0	1	0	3	0
Infiltrate, neutrophil	1	0	4	2	8	9	10	6	4
Minimal	1	–	4	1	5	9	5	5	1
Mild	0	–	0	1	3	0	5	1	3
Necrosis, single cell	0	0	1	1	5	3	10	6	4
Minimal	–	–	1	1	5	3	10	6	3
Moderate	–	–	0	0	0	0	0	0	1
Liver									
Degeneration/Necrosis, hepatocellular	0	0	NE	NE	0[m]	0	0	0	5
Mild	–	–	–	–	–	–	–	–	4
Moderate	–	–	–	–	–	–	–	–	1
Infiltrate, neutrophil	0	0	NE	NE	0	0	0	1	1
Minimal	–	–	–	–	–	–	–	1	1
Vacuolation, hepatocellular, generalized	0	0	NE	NE	0	0	0	1	5
Minimal			–	–				1	
Moderate	–	–	–	–	–	–	–	–	5

2.6.7.7C Repeat-Dose Toxicity: A 6-Month Oral Gavage Toxicity Study of MLN9708 With a 2-Week Recovery Period in Sprague-Dawley Rats (continued)

A 7-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period									
Daily dose (mg/kg)[a]	0 (Control)		0.2		0.4		0.8/0.6[b]		
Number of animals (main study and early death)	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10 (MS)	F:5 (ED)
Lymph Node, Mesenteric									
Necrosis, lymphoid	0	0	1	1	3	3	6	7	5
Minimal	–	–	1	1	3	3	6	6	2
Mild	–	–	–	–	–	–	–	1	3
Depletion, lymphoid	1	0	2	0	5	0	7	0	0
Minimal	1	–	2	–	2	–	6	–	–
Mild	–	–	–	–	3	–	1	–	–
Infiltrate, neutrophil	0	0	0	0	0	0	3	0	0
Minimal	–	–	–	–	–	–	3	–	–
Mammary Gland									
Hyperplasia, lobuloalveolar (with or without atypia)	N/A	4	N/A	NE	N/A	2[n]	N/A	4	4
Minimal	–	4	–	–	–	2	–	4	4
Secretion	N/A	2	N/A	NE	N/A	2	N/A	4	1
Minimal	–	2	–	–	–	2	–	4	1
Marrow, Sternum									
Hypocellular	0	1	NE	1	0	3	2	1	5
Minimal	–	1	–	1	–	3	2	1	5
Necrosis, single cell	0	0	NE	0	0	0	0	0	3
Minimal	–	–	–	–	–	–	–	–	3

2.6.7.7C Repeat-Dose Toxicity: A 6-Month Oral Gavage Toxicity Study of MLN9708 With a 2-Week Recovery Period in Sprague-Dawley Rats (continued)

A 7-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period									
Daily dose (mg/kg)[a]	0 (Control)		0.2		0.4		0.8/0.6[b]		
Number of animals (main study and early death)	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10 (MS)	F:5 (ED)
Salivary gland									
Increased mucin	0	0	0	0	6	4	8	4	5
Minimal	–	–	–	–	5	4	2	3	2
Mild	–	–	–	–	1	–	3	1	2
Moderate	–	–	–	–	–	–	3	–	1
Spleen									
Depletion, lymphoid	0	0	0	NE	0	0	0	0	3
Minimal	–	–	–	–	–	–	–	–	2
Mild	–	–	–	–	–	–	–	–	1
Infiltrate, neutrophil	4	2	1	NE	7	3	10	5	0
Minimal	4	2	1	–	5	3	3	4	–
Mild	–	–	–	–	2	–	7	1	–
Necrosis, redpulp	0	0	0	NE	0	0	0	0	4
Minimal	–	–	–	–	–	–	–	–	3
Mild	–	–	–	–	–	–	–	–	1
Stomach, glandular									
Inflammation subacute	0	0	0	NE	3	0	6	1	0
Minimal	–	–	–	–	3	–	5	1	–
Moderate	–	–	–	–	–	–	1	–	–
Erosion	0	0	0	NE	0	1	3	0	0
Minimal	–	–	–	–	–	1	2	–	–
Mild	–	–	–	–	–	–	1	–	–

2.6.7.7C Repeat-Dose Toxicity: A 6-Month Oral Gavage Toxicity Study of MLN9708 With a 2-Week Recovery Period in Sprague-Dawley Rats (continued)

A 7-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period									
Daily dose (mg/kg)[a]	0 (Control)		0.2		0.4		0.8/0.6[b]		
Number of animals (main study and early death)	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10 (MS)	F:5 (ED)
Stomach, Nonglandular									
Ulceration	0	0	NE	NE	NE	0	0	1	0
Minimal	–	–	–	–	–	–	–	1	–
Inflammation subacute	0	0	NE	NE	NE	0	0	1	0
Minimal	–	–	–	–	–	–	–	1	–
Thymus									
Depletion, lymphoid	0	0	NE	NE	0	0	1	0	0
Minimal	–	–	–	–	–	–	1	–	–
Testes									
Retention spermatids	0	N/A	NE	N/A	0	N/A	1	N/A	N/A
Minimal	–	–	–	–	–	–	1	–	–
Tongue									
Ulceration	0	0	NE	NE	NE	0	0	0	1
Mild	–	–	–	–	–	–	–	–	1

2.6.7.7C Repeat-Dose Toxicity: A 6-Month Oral Gavage Toxicity Study of MLN9708 With a 2-Week Recovery Period in Sprague-Dawley Rats (continued)

A 7-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Sprague-Dawley Rat With a 14-Day Recovery Period								
Daily dose (mg/kg)[a]	0 (Control)		0.2		0.4		0.8/0.6[b]	
Recovery evaluations								
Number of animals (recovery)	M:5	F:5	M:5	F:5	M:3	F:5	M:5	F:0
Body weight and food consumption	All findings were reversed or reversing during the recovery period.							
Clinical pathology	All findings were reversed or reversing during the recovery period.							
Recovery Necropsy (Week 28)								
Organ weights (recovery necropsy)	All findings were reversed or reversing during the recovery period.							
Microscopic findings								
Ileum								
Necrosis, single cell	0	0	NE	NE	0	0	1	N/A
Minimal	–	–	–	–	–	–	1	–
Lymph Node, Mesenteric								
Depletion, lymphoid	0	0	NE	NE	0	0	3	N/A
Minimal	–	–	–	–	–	–	2	–
Mild	–	–	–	–	–	–	1	–
Marrow, Sternum								
Hypocellular	0	0	NE	NE	0	0	1	N/A
Minimal	–	–	–	–	–	–	1	–
Salivary Gland								
Increased mucin	0	0	NE	0	NE	1	0	N/A
Minimal	–	–	–	–	–	1	–	–
Spleen								
Infiltrate, neutrophil	2	0	NE	NE	0	0	1	N/A
Minimal	2	–	–	–	–	–	1	–

*p < 0.05, **p < 0.01 (Dunnett test); + = findings observed; – = no noteworthy findings; APTT = activated partial thromboplastin time; AUC_{24} = area under the plasma concentration-time curve from the time 0 to 24 hours; AUC_{168} = area under the plasma concentration-time curve from the time 0 to 168 hours; BASO = basophil; Cmax = maximum observed plasma concentration; ED = early death; F = female(s); GLP = Good Laboratory Practice; LYMPH = lymphocyte; M = male(s); MONO = monocyte; MS = main study; MTD = maximum tolerated dose; N/A = not applicable; NE = not evaluated; NEUT = neutrophil; NOAEL = no observed adverse effect level; PO = oral(ly); QW = once weekly; SWI = sterile water for injection; USP = United States Pharmacopeia; WBC = white blood cell.

a Doses are in terms of mg/kg ixazomib.

b Dose for females was reduced to 0.6 mg/kg starting with Cycle 3 due to early euthanasia/mortality.

c n = 15 animal sex/group, but blood was collected from 3/sex/group/time point.

d Two females were found dead on Days 1 or 2 and were subsequently replaced. The cause of these deaths could not be determined due to lack of collection of microscopic data.

e These deaths were not considered test-article related and therefore microscopic findings for these animals are not discussed here.

f The cause of death as determined from the microscopic evaluation was multifactorial (liver, intestine, and/or lymphoid lesions).

g At the end of the dosing period (Week 26).

h For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not percent differences).

i +/– represents presence or absence of findings.

j Number of findings in examined tissues.

k If no findings were present in higher-dose animals, then tissues were not examined in the next lower dose. All tissues not examined for this reason have been marked “NE.”

l Includes duodenum, ileum, and rectum.

m Tissue examined from 1 animal only.

n Tissue examined from 9 animals only.

2.6.7.7D Repeat-Dose Toxicity: A Repeat-Dose Intravenous Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats

Species/strain:	Rat/ Sprague-Dawley	Duration of dosing:	32 days (2 cycles of BIW dosing for 2 weeks; separated by a 10-day nondosing period)	Test article:	Ixazomib
Initial age:	8 - 9 weeks	Duration of recovery period:	14 days	Report:	4.2.3.2-7, and 4.2.3.2-8
Date of first dose:	[redacted]	Day of first dose (Day 0 or Day 1):	Day 1	GLP compliance:	Yes
Method of administration:	IV bolus				
Vehicle/formulation:	[redacted] and 1% propylene glycol, [redacted]				
Special features:	Pharmacodynamic analysis (20S proteasome activity, assessed by fluorometry) was done in a separate, non-GLP-compliant study (Report 4.2.3.2-8).				
Study endpoint:	STD10 = 0.45 mg/kg (females)				

A Repeat-Dose Intravenous Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats

Daily dose (mg/kg)[a]	0 (Control)		0.15		0.30		0.45[b]; 0.50[c]	0.22[b]; 0.45[c]
Number of animals (toxicokinetic)	M:3	F:3	M:9	F:9	M:9	F:9	M:9	F:9
Toxicokinetics[d]								
C_{max} (ng/mL)								
Day 1	N/A		125		318		511[e]	237
Day 11	N/A		148		325		529	220
Day 22	N/A		173		339		812	483
AUC_{24} (ng·hr/mL)								
Day 1	N/A		291		603		940[e]	450
Day 11	N/A		369		645		853	582
Day 22	N/A		350		660		1260	1310

2.6.7.7D Repeat-Dose Toxicity: A Repeat-Dose Intravenous Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats (continued)

A Repeat-Dose Intravenous Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats								
Daily dose (mg/kg)[a]	0 (Control)		0.15		0.30		0.45[b]; 0.50[c]	0.22[b]; 0.45[c]
Number of animals (pharmacodynamic)	M:3	F:3	M:9	F:9	M:9	F:9	M:9	F:9
Pharmacodynamics								
E_{max} (I%)								
Day 1	N/A	N/A	70.8	52.9	78.9	70.6	81.4	69.4
Day 11	N/A	N/A	75.1	62.2	80.2	73.3	76.5	73.7
Day 22	N/A	N/A	78.8	63.0	79.4	73.1	78.9	72.2
AUE_{24} (hr·I%)								
Day 1	N/A	N/A	1070	277	1090	695	1320	657
Day 11	N/A	N/A	899	577	1090	602	1210	858
Day 22	N/A	N/A	1260	368	1110	565	1120	1040
Noteworthy findings								
Number of animals (main and recovery)	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15
Died or euthanized moribund	–	–	1[f]	1[f]	–	1[f]	–	2[g]
Body weight (g)[h]	429.20	278.05	–	–	–7.5*	–	–12.9*	–
Food consumption (g)[h]								
Days 1 - 8	29.6	22.7	–	–	–	–	–17.9*	–
Days 22 - 29	34.0	23.0	–	–	–14.7*	–	–20.6*	–15.7*
Days 29 - 32	38.3	26.6	–	–	–13.1*	–	–	–
Clinical observations[i]								F:_2 (ED) F:_13 (MS)
Dry red material	–	–	+[j]	+[j]	+	+	+	+ +
Lacrimation	–	–	–	–	–	–	–	+ +
Soft feces	–	–	–	–	–	–	+	+ +

2.6.7.7D Repeat-Dose Toxicity: A Repeat-Dose Intravenous Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats (continued)

A Repeat-Dose Intravenous Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats									
Daily dose (mg/kg)[a]	0 (Control)		0.15		0.30		0.45[b]; 0.50[c]	0.22[b]; 0.45[c]	
Number of animals (main and recovery)	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15	
Hematology[h]									
EOS, abs ($10^3/\mu L$)	0.073	0.067	–	–	–88*	–64*	–97*	–88*	
MON, abs ($10^3/\mu L$)	0.127	0.096	–	–	–	–	–52*	–52*	
NEUT, abs ($10^3/\mu L$)	0.952	0.647	–	–	–	–	–32	–	
PLT ($10^3/\mu L$)	1205.7	1137.5	–37*	–35*	–57*	–61*	–60*	–79*	
Clinical chemistry[h]									
ALP (U/L)	161.3	84.6	–	–	–	–32*	–43*	–39*	
ALT (U/L)	35.5	29.0	–	–	–42*	–40*	–44*	–43*	
AST (U/L)	170.5	133.5	–	–	–35*	–21*	–33*	–39*	
BUN (mg/dL)	17.0	17.0	–	–14*	–25*	–23*	–29*	–27*	
Primary Necropsy (Day 33)									
Number of animals (main study and early deaths)	M:10	F:10	M:9	F:9	M:10	F:10	M:10	F:2 (ED)	F:8 (MS)
Organ weights (g)[k]									
Liver	12.63	7.46	–	+22*	–	+39*	+12	+55*	
Thymus	0.3672	0.3333	–	–	–73*	–69*	–84*	–72*	
Thyroid gland	0.0257	0.0174	–	+26*	–	+24*	–	+37*	
Macroscopic findings[l]									
Stomach									
Distended	–	–	–	–	2	2	4	–	5
Thymus									
Small	–	–		–	6	4	9	–	7

2.6.7.7D Repeat-Dose Toxicity: A Repeat-Dose Intravenous Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats (continued)

A Repeat-Dose Intravenous Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats									
Daily dose (mg/kg)[a]	0 (Control)		0.15		0.30		0.45[b]; 0.50[c]	0.22[b]; 0.45[c]	
Number of animals (primary necropsy and early deaths)	M:10	F:10	M:9	F:9	M:10	F:10	M:10	F:2 (ED)	F:8 (MS)
Microscopic findings[l]									
Bone marrow[m]									
Hypocellularity	0	0	0	0	2	3	10	1	8
Minimal	–	–	–	–	1	2	2	–	1
Mild	–	–	–	–	–	1	2	–	1
Moderate	–	–	–	–	1	–	5	1	4
Marked	–	–	–	–	–	–	1	–	2
Dorsal root ganglia[n]									
Chromatolysis, multifocal	0	0	0	0	3	3	4	1	3
Minimal	–	–	–	–	3	3	3	–	3
Mild	–	–	–	–	–	–	1	1	–
Liver									
Hepatocellular hypertrophy	0	0	0	0	0	2	0	0	6
Minimal	–	–	–	–	–	2	–	–	5
Mild	–	–	–	–	–	–	–	–	1
Hepatocellular vacuolation	0	0	0	0	0	3	0	0	1
Minimal	–	–	–	–	–	3	–	–	1

2.6.7.7D Repeat-Dose Toxicity: A Repeat-Dose Intravenous Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats (continued)

A Repeat-Dose Intravenous Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats									
Daily dose (mg/kg)[a]	0 (Control)		0.15		0.30		0.45[b]; 0.50[c]	0.22[b]; 0.45[c]	
Number of animals (primary necropsy and early deaths)	M:10	F:10	M:9	F:9	M:10	F:10	M:10	F:2 (ED)	F:8 (MS)
Intestine[o]									
Glandular epithelial hyperplasia	0	0	0	0	10	6	9	0	9
Minimal	–	–	–	–	10	6	8	–	6
Mild	–	–	–	–	–	–	1	–	3
Sciatic nerve									
Degeneration	0	1	0	0	0	0	5	0	1
Minimal	–	1	–	–	–	–	5	–	1
Sural nerve									
Degeneration	0	0	0	0	1	0	4	0	0
Minimal	–	–	–	–	1	–	4	–[p]	–
Thymus									
Depletion, lymphocytic	0	0	0	0	6	10	9	0	8
Minimal	–	–	–	–	–	4	–	–	–
Mild	–	–	–	–	2	1	–	–	–
Moderate	–	–	–	–	3	3	–	–	1
Marked	–	–	–	–	1	2	9	–	7

2.6.7.7D Repeat-Dose Toxicity: A Repeat-Dose Intravenous Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats (continued)

A Repeat-Dose Intravenous Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats									
Daily dose (mg/kg)[a]	0 (Control)		0.15		0.30		0.45[b]; 0.50[c]	0.22[b]; 0.45[c]	
Number of animals (primary necropsy and early deaths)	M:10	F:10	M:9	F:9	M:10	F:10	M:10	F:2 (ED)	F:8 (MS)
Bone marrow smear analysis[q]									
Miscellaneous cells									
Lymphocyte	184.0	202.4	–	–	135.7	146.0	126.6	149.5	
Metarubricyte	67.8	78.1	–	–	117.8	115.2	130.0	136.8	
Rubricyte	80.7	75.4	–	–	131.0	119.2	157.2	142.6	
Smear cellularity	–	–	–	–	Decreased	Decreased	Decreased	Decreased	
Total myeloid cell count	156.8	132.1	–	–	104.8	107.4	61.6	48.4	
Eosinophil	13.1	19.5	–	–	2.8	6.1	1.8	2.0	
Myelocyte	9.8	6.8	–	–	4.5	–	3.4	3.0	
Metamyelocyte	14.4	7.6	–	–	5.3	–	4.9	2.8	
Band neutrophil	21.5	13.3	–	–	–	–	13.5	–	
Segmented neutrophil	88.6	78.6	–	–	68.4	63.5	30.4	23.8	
Total erythroid cells	149.8	155.3	–	–	251.5	237.1	288.5	282.8	
M:E	1.1	0.9	–	–	0.4	0.5	0.3	0.2	

2.6.7.7D Repeat-Dose Toxicity: A Repeat-Dose Intravenous Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats (continued)

A Repeat-Dose Intravenous Toxicity Study of MLN2238 With a 14-Day Recovery Period in Sprague-Dawley Rats								
Daily dose (mg/kg)[a]	0 (Control)		0.15		0.30		0.45[b]; 0.50[c]	0.22[b]; 0.45[c]
Recovery evaluations								
Number of animals (recovery)	M:5	F:5	M:5	F:5	M:5	F:4	M:5	F:5
Recovery necropsy (Day 47)								
Organ weights (g)[g]								
Liver	13.42	7.80	–	–	–	–	–	+21*
Bone marrow smear analysis[o]								
Total myeloid cell count	–	136.4	–	–	–	–	–	118.5
Total erythroid cell count	–	144.2	–	–	–	–	–	215.8
M:E	–	1.0	–	–	–	–	–	0.5
Miscellaneous cells								
Lymphocyte	–	208.8	–	–	–	156.5	–	156.3

* $p \leq 0.05$ (Dunnett test); – = no noteworthy findings; abs = absolute; ALP = alkaline phosphatase; ALT = alkaline aminotransferase; AST = aspartate aminotransferase; AUC_{24} = area under the plasma concentration-time curve from the time 0 to 24 hours; AUE_{24} = area under the effect-time curve from time 0 to 24 hours; BIW = twice weekly; BUN = blood urea nitrogen; C_{max} = maximum observed plasma concentration; ED = early death; Emax = maximum effect; EOS = eosinophil; F = female(s); GLP = Good Laboratory Practice; I% = percentage of proteasome inhibition; IV = intravenous(ly); M = male(s); M:E = myeloid-to-erythroid ratio; MON = monocyte; MS = main study; N/A = not applicable; NE = not examined; NEUT = neutrophil; PLT = platelet; STD10 = severely toxic dose in 10% of animals administered test article.

a Female toxicokinetic (TK) satellite rats were dosed at 0.45 mg/kg (Group 4) before the remainder of the rats. High mortality resulted and no other rats in Group 4 were dosed; Group 5, female rats dosed at 0.22 mg/kg, was formed. Because of a lack of toxicity, Group 4 male rats, dosed at 0.45 mg/kg during Cycle 1, were dosed at 0.50 mg/kg during Cycle 2, and Group 5 female rats, dosed at 0.22 mg/kg during Cycle 1, were dosed at 0.45 mg/kg during Cycle 2.

b Cycle 1.

c Cycle 2.

d n = 9/sex/group, but blood was collected for TK from 3/sex/group/time point. Results for the 0.15 and 0.30 mg/kg dose groups were combined for both sexes, because of a lack of sex-related differences.

e Includes females that were dosed with 0.45 mg/kg on Day 1 only.

f Deaths were not considered test article related. Deaths were considered procedure related, and were attributed to blood collection error on Day 12.

g One death was not test article related, but was attributed to blood collection error on Day 12. The other death was considered test article related. Animal was found dead on Day 24; a definitive cause of death was not determined for this female, as autolysis prevented full analysis of the tissues.

h At the end of the dosing period (Week 5). For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

i +/- indicates presence or absence of finding.

j This finding occurred in extremely low incidence in the 0.15 mg/kg group; therefore, relation to the test article could not be determined.

k Both absolute and relative weights differed from controls in the direction indicated. For controls, absolute organ weight group means are shown; for ixazomib-dosed groups, percent differences from controls are shown. Organ weights evaluated in primary and recovery necropsy animals only; results do not include early death animals.

l Numbers represent animals with findings present in tissues examined.

m Includes bone marrow from femur and sternum. When findings differed in severity amongst sections, the highest level of severity is presented.

n Includes cervical and lumbar dorsal root ganglias (DRGs). When findings differed in severity amongst sections, the highest level of severity is presented.

o Includes all sections of the intestine. When findings differed in severity amongst sections, the highest level of severity is presented.

p Sural nerve was examined from only 1 of the early death high-dose females.

q The number represents the group mean. Bone marrow smears were taken from primary and recovery animals only; results do not include early death animals.

2.6.7.7E Repeat-Dose Toxicity: A 5-Cycle Intravenous (Bolus) Toxicity and Toxicokinetic Study of MLN9708 in the Sprague-Dawley Rat

Species/Strain:	Rat/ Sprague-Dawley, Crl:CD	Duration of dosing:	94 days (5 cycles of BIW dosing for 2 weeks, separated by a 10-day nondosing observation period)	Test article:	Ixazomib citrate
Initial age:	7 weeks	Duration of recovery period:	N/A	Report:	4.2.3.2-9
Date of first dose:	[redacted]	Day of first dose (Day 0 or Day 1):	Day 0	GLP compliance:	Yes
Method of administration:	Intravenous (bolus) injection				
Vehicle/formulation:	0.9% Sodium Chloride For Injection, USP				
Special features:	None				
Study endpoint:	MTD = 0.15 mg/kg				

A 5-Cycle Intravenous (Bolus) Toxicity and Toxicokinetic Study of MLN9708 in the Sprague-Dawley Rat

Daily dose (mg/kg)[a]	0 (Control)		0.05		0.15		0.30	
Number of animals (toxicokinetic)	M: 6	F: 6	M: 18	F: 18	M: 18	F: 18	M: 18	F: 18
Toxicokinetics								
C_{max} (ng/mL)								
Day 0	N/A		28.1		101		228	
Day 94	N/A		44.7		172		283	
AUC_{24} (ng·hr/mL)								
Day 0	N/A		103		283		580	
Day 94	N/A		184		454		942	

2.6.7.7E Repeat-Dose Toxicity: A 5-Cycle Intravenous (Bolus) Toxicity and Toxicokinetic Study of MLN9708 in the Sprague-Dawley Rat (continued)

A 5-Cycle Intravenous (Bolus) Toxicity and Toxicokinetic Study of MLN9708 in the Sprague-Dawley Rat										
Daily dose (mg/kg)[a]	0 (Control)		0.05		0.15		0.30			
Number of animals (main study)	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10		F: 10	
Noteworthy findings										
Died or euthanized moribund	–	–	–	–	–	–	1[b]		2[c]	
Body weight (g)[d]	552	289	–4.3	–1.0	–2.5	+1.0	–12.7**		+1.0	
Food consumption (g/day)[d]	29	19	–3.4	–5.3	–10.3	0.0	–17.2**		+5.3	
Clinical observations[e]							M:1 (ED)	M:9 (MS)	F:2 (ED)	F:8 (MS)
Body cool to the touch	–	–	–	–	–	–	+	–	+	–
Brown material, anogenital area	–	–	–	–	–	–	+	+	+	+
Clear discharge from the eyes	–	–	–	+	–	+	–	+	+	+
Clear material[f]	+	–	–	–	+	+	+	+	–	+
Extremities cool to the touch	–	–	–	–	–	–	–	–	+	+
Hypoactivity	–	–	–	–	–	–	+	–	–	–
Mucoid feces	–	–	–	–	–	–	+	+	–	–
Rectal mucosal exudate	–	–	–	–	–	–	–	+	–	–
Shallow respiration	–	–	–	–	–	–	+	–	–	–
Soft feces	–	–	–	–	–	–	+	+	+	+
Yellow material[g]	–	+	+	+	+	+	+	+	+	+
Hematology[d]										
EOS, abs ($10^3/\mu L$)	0.10	0.07	+30.0	–14.3	–50.0*	–28.6**	–70.0**		–71.4**	
HDW (g/dL)	2.58	2.27	+20.9**	+10.6*	+26.4**	+19.8**	+36.0**		+36.6**	
MONO, abs ($10^3/\mu L$)	0.16	0.10	+18.8	0.0	+25.0	–10.0	+12.5		+10.0	
MPV (fL)	6.20	6.21	+2.7	–0.3	+14.5**	+10.8**	+25.2**		+29.3**	

2.6.7.7E Repeat-Dose Toxicity: A 5-Cycle Intravenous (Bolus) Toxicity and Toxicokinetic Study of MLN9708 in the Sprague-Dawley Rat (continued)

A 5-Cycle Intravenous (Bolus) Toxicity and Toxicokinetic Study of MLN9708 in the Sprague-Dawley Rat								
Daily dose (mg/kg)[a]	0 (Control)		0.05		0.15		0.30	
Number of animals (main study)	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
NEUT, abs (10^3/μL)	0.88	0.64	+64.8	–12.5	+168.2*	+15.6	+192.0*	+70.3
PLT (10^3/μL)	1039	981	–14.2*	–4.3	–40.1**	–34.5**	–40.9**	–50.3**
RDW (%)	13.4	12.9	0.0	0.0	+3.0	+8.5	+19.4**	+35.7**
RETIC, abs (10^3/μL)	154.3	148.7	+4.2	–6.5	+10.0	+37.0	+17.0	+65.6**
WBC (10^3/μL)	7.70	5.30	+33.0	+18.3	+46.8*	+29.2	+5.8	+11.1
Coagulation[d]								
APTT (seconds)	19.7	17.0	0.0	+1.2	+6.6	+6.5	+8.1	+14.1*
FIB (mg/dL)	229.6	171.9	+0.8	+4.0	+8.5	+6.8	+43.9	+16.2*
PT (seconds)	17.8	17.1	–3.9%	0.0	–1.1	+5.3*	+2.8	+6.4*
Clinical chemistry[d]								
ALB (g/dL	4.4	5.0	0.0	+6.0	+2.3	+10.0**	0.0	+2.0
ALP (U/L)	87	40	–3.4	+7.5	–17.2	–12.5	–21.8	+12.5
BICARB (mmol/L)	26	24	–3.8	–4.2	–3.8	–8.3	0.0	0.0
BIL, direct (mg/dL)	0.04	0.04	0.0	0.0	0.0	+25.0	0.0	+50.0*
BIL, indirect (mg/dL)	0.19	0.19	0.0	+15.8	0.0	+5.3	0.0	+10.5
TBIL (mg/dL)	0.22	0.23	+4.5	+13.0	+4.5	+13.0	+4.5	+17.4
Calcium (mg/dL)	11.5	11.6	0.0	+3.4	+1.7	+5.2*	–2.6	–2.6
CHOL (mg/dL)	64	67	–14.1	+13.4	–10.9	+26.9	–32.8	–35.8*
Chloride (mEq/L)	103	104	+1.0	0.0	+1.9	0.0	–1.9	0.0
GLOB (g/dL)	2.3	2.2	–8.7	–4.5	+4.3	–9.1	–8.7	–22.7**
Glucose (mg/dL)	116	112	+3.4	+5.4	+10.3	+12.5	+19.8**	+24.1**
TP (g/dL)	6.7	7.1	–3.0	+4.2	+3.0	+5.6	–4.5	–4.2
TRIG (mg/dL)	65	33	+15.4	+24.2	–4.6	+60.6*	+15.4	+84.8**

2.6.7.7E Repeat-Dose Toxicity: A 5-Cycle Intravenous (Bolus) Toxicity and Toxicokinetic Study of MLN9708 in the Sprague-Dawley Rat (continued)

A 5-Cycle Intravenous (Bolus) Toxicity and Toxicokinetic Study of MLN9708 in the Sprague-Dawley Rat										
Daily dose (mg/kg)[a]	0 (Control)		0.05		0.15		0.30			
Number of animals (main study)	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10		F: 10	
Urinalysis[d]										
Calcium (mg/dL)	2.9	12.0	–51.7	–45.8	–51.7	–58.3**	+3.4		–87.5**	
Chloride (mEq/L)	41	37	–36.6	0.0	–39.0	–24.3	–63.4		–78.4*	
CRE clearance (mL/min)	4.50	1.73	+9.1	–4.0	–0.4	+13.3	–17.6		+19.1	
Osmolality (mOsm/kg)	1012	669	–52.9*	–13.8	–25.9	–21.7	–20.1		–47.7*	
PHOS (mg/dL)	212.0	200.6	–39.5	–14.8	–36.6	–40.7	+60.5		–65.4**	
Potassium (mEq/L)	163.59	130.60	–37.1	–15.1	–25.2	–35.0	+32.7		–60.7	
Sodium (mEq/L)	48	44	–18.8	+11.4	–6.3	–6.8	–14.6		–68.2*	
Total volume (mL)	8.7	6.4	+92.0*	–14.1	+48.3	+95.3	–9.2		+187.5**	
Primary necropsy (Day 95)										
Number of animals (main study and early death)	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 1 (ED)	M: 9 (MS)	F: 2 (ED)	F: 8 (MS)
Organ Weights (g)[d, h, i]										
Liver	14.92	7.51	+2.8	+6.3	+3.2	+19.6**	+2.0		+44.3**	
Kidneys	3.87	1.98	–3.1	+8.1	–2.8	+15.7**	–1.8		+16.2**	
Thymus	0.2258	0.2353	+22.6*	+10.2	+2.7	+0.9	–78.5**		–71.6**	
Macroscopic findings[j, k]										
Thymus										
Small	–	–	–	–	–	–	1	6	–	4

2.6.7.7E Repeat-Dose Toxicity: A 5-Cycle Intravenous (Bolus) Toxicity and Toxicokinetic Study of MLN9708 in the Sprague-Dawley Rat (continued)

A 5-Cycle Intravenous (Bolus) Toxicity and Toxicokinetic Study of MLN9708 in the Sprague-Dawley Rat										
Daily dose (mg/kg)[a]	0 (Control)		0.05		0.15		0.30			
Number of animals (main study and early death)	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 1 (ED)	M: 9 (MS)	F: 2 (ED)	F: 8 (MS)
Microscopic findings[d, j]										
Adrenal cortex										
Zona glomerulosa hypertrophy	0	0	0	1	0	0	0	2	1	1
Minimal	–	–	–	1	–	–	–	1	–	1
Mild	–	–	–	–	–	–	–	1	–	–
Moderate	–	–	–	–	–	–	–	–	1	–
Bone marrow[l],										
Hypocellular	0	0	1	0	2	1	1	6	2	6
Minimal	–	–	1	–	2	1	–	6	1	6
Mild	–	–	–	–	–	–	–	–	1	–
Moderate	–	–	–	–	–	–	1	–	–	–
Dorsal root ganglia[m]										
Chromatolysis	0	0	0	0	1	1	0	4	0	2
Minimal	–	–	–	–	1	1	–	3	–	2
Mild	–	–	–	–	–	–	–	1	–	–
Glandular stomach										
Acute inflammation	0	0	1	0	0	0	0	2	0	0
Minimal	–	–	1	–	–	–	–	1	–	–
Moderate	–	–	–	–	–	–	–	1	–	–
Epithelial hyperplasia	0	0	0	0	0	0	0	2	0	0
Minimal	–	–	–	–	–	–	–	2	–	–
Single cell necrosis	0	0	0	0	0	0	0	1	0	0
Minimal	–	–	–	–	–	–	–	1	–	–

2.6.7.7E Repeat-Dose Toxicity: A 5-Cycle Intravenous (Bolus) Toxicity and Toxicokinetic Study of MLN9708 in the Sprague-Dawley Rat (continued)

A 5-Cycle Intravenous (Bolus) Toxicity and Toxicokinetic Study of MLN9708 in the Sprague-Dawley Rat										
Daily dose (mg/kg)[a]	0 (Control)		0.05		0.15		0.30			
Number of animals (main study and early death)	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 1 (ED)	M: 9 (MS)	F: 2 (ED)	F: 8 (MS)
Microscopic findings[d, j] (continued)										
Intestine[n]										
Acute inflammation	0	0	0	0	0	0	1	7	1	6
Minimal	–	–	–	–	–	–	–	5	–	6
Mild	–	–	–	–	–	–	1	2	1	0
Epithelial hyperplasia	0	0	0	0	0	0	1	2	0	0
Minimal	–	–	–	–	–	–	1	2	–	–
Single cell necrosis	0	0	0	1	0	0	1	6	1	2
Minimal	–	–	–	1	–	–	–	5	–	2
Mild	–	–	–	–	–	–	–	1	–	–
Moderate	–	–	–	–	–	–		–	1	–
Marked	–	–	–	–	–	–	1	–	–	–
Kidneys										
Mineralization, increased	0	0	0	0	0	0	0	0	1	2
Minimal	–	–	–	–	–	–	–	–	–	2
Mild	–	–	–	–	–	–	–	–	1	–
Renal tubular regeneration	0	0	0	0	0	0	0	1	0	0
Moderate	–	–	–	–	–	–	–	1	–	–
Tubular dilatation	0	0	0	0	0	0	0	7	0	1
Minimal	–	–	–	–	–	–	–	4	–	1
Mild	–	–	–	–	–	–	–	3	–	–
Lacrimal gland										
Single cell necrosis	0	1	1	0	0	0	0	6	0	0
Minimal	–	1	1	–	–	–	–	4	–	–
Mild	–	–	–	–	–	–	–	1	–	–
Moderate	–	–	–	–	–	–	–	1	–	–

2.6.7.7E Repeat-Dose Toxicity: A 5-Cycle Intravenous (Bolus) Toxicity and Toxicokinetic Study of MLN9708 in the Sprague-Dawley Rat (continued)

A 5-Cycle Intravenous (Bolus) Toxicity and Toxicokinetic Study of MLN9708 in the Sprague-Dawley Rat										
Daily dose (mg/kg)[a]	0 (Control)		0.05		0.15		0.30			
Number of animals (main study and early death)	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 1 (ED)	M: 9 (MS)	F: 2 (ED)	F: 8 (MS)
Microscopic findings[d, j] (continued)										
Lymph node[o]										
Acute inflammation	0	0	0	0	0	0	0	3	0	0
Minimal	–	–	–	–	–	–	–	2	–	–
Mild	–	–	–	–	–	–	–	1	–	–
Single cell necrosis	0	2	4	1	4	4	1	7	2	5
Minimal	–	2	3	1	4	4	–	4	1	3
Mild	–	–	1	–	–	–	–	3	1	2
Moderate	–	–	–	–	–	–	1	–	–	–
Peyer's patches										
Lymphoid depletion	0	0	0	1	0	0	0	2	0	0
Minimal	–	–	–	1	–	–	–	2	–	–
Single cell necrosis	4	6	5	7	5	6	1	5	2	7
Minimal	4	6	5	7	4	4	–	4	–	4
Mild	–	–	–	–	1	2	–	1	1	2
Moderate	–	–	–	–	–	–	1	–	1	1
Thymus										
Lymphoid depletion	1	1	0	1	3	2	1	9	1	7
Minimal	1	1	–	1	3	2	–	–	1	–
Mild	–	–	–	–	–	–	1	–	–	1
Moderate	–	–	–	–	–	–	–	8	–	6
Severe	–	–	–	–	–	–	–	1	–	–

*p < 0.05, ** p < 0.01 (Dunnett test); + = findings observed; – = no noteworthy findings; abs = absolute; ALB = albumin; ALP = alkaline phosphatase; APTT = activated partial thromboplastin time; AUC_{24} = area under the plasma concentration-time curve from the time 0 to 24 hours; BICARB = bicarbonate; BIL = bilirubin; BIW = twice weekly; CHOL = cholesterol; Cmax = maximum observed plasma concentration; CRE = creatinine; ED = early death; EOS = eosinophil; F = female(s); FIB = fibrinogen; GLOB = globulin; GLP = Good Laboratory Practice; HDW = hemoglobin distribution width; M = male(s); MONO = monocyte; MPV = mean platelet volume; MS = main study; MTD = maximum tolerated dose; N/A = not applicable; NEUT = neutrophil; PHOS = phosphorus; PLT = platelet; PT = prothrombin time; RDW = red blood cell distribution width; RETIC = reticulocyte; TBIL = total bilirubin; TP = total protein; TRIG = triglyceride; USP = United States Pharmacopeia; WBC = white blood cell.

a Doses are presented as mg/kg ixazomib.

b Animal was euthanized on Day 85 following adverse clinical signs.

c Animals were found dead on Days 86 and 92 following adverse clinical signs.

d At the end of the dosing period (Week 13). For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not percent differences).

e +/– indicates presence or absence of finding.

f Noted on forelimbs, mouth, and/or ventral neck.

g Noted on anogenital area, base of tail, hind limbs, urogenital area, and/or ventral trunk.

h Both absolute and relative weights differed from controls in the direction indicated. Number indicates percent difference for the absolute organ weights.

i Per protocol, organ weights were not measured for unscheduled deaths.

j Numbers indicate total incidence.

k Week 13, except for unscheduled deaths.

l Includes findings in both femur and sternum. When severity of findings differed amongst sites, the highest level of severity is presented.

m Includes findings in both cervical and lumbar dorsal root ganglias (DRGs). When severity of findings differed amongst sites, the highest level of severity is presented.

n Includes all sections of the intestine. When severity of findings differed amongst sites, the highest level of severity is presented.

o Includes findings in axillary, mandibular, mesenteric, and popliteal lymph nodes. When severity of findings differed amongst lymph nodes, the highest level of severity is presented.

2.6.7.7F Repeat-Dose Toxicity: MLN2238: A Repeat-Dose Toxicity Study of MLN2238 Administered via Oral Gavage in the Beagle Dog With a 14-Day Recovery Period

Species/strain:	Dog/beagle	Duration of dosing:	11 Days (1 cycle of BIW dosing for 2 weeks)	Test article:	Ixazomib
Initial age:	7 - 8 months	Duration of recovery period:	14 days (25 days for Group 4)	Report:	4.2.3.2-11
Date of first dose:	[redacted]	Day of first dose (Day 0 or Day 1):	Day 1	GLP compliance:	Yes

Method of administration: Oral gavage
Vehicle/formulation: [redacted] and 1% (w:w) propylene glycol, [redacted]
Special features: Pharmacodynamic analysis (20S proteasome activity, assessed by fluorometry) was done in a separate, non-GLP-compliant study (Report4.2.3.2-12).
Study endpoint: HNSTD = 0.15 mg/kg

MLN2238: A Repeat-Dose Toxicity Study of MLN2238 Administered via Oral Gavage in the Beagle Dog With a 14-Day Recovery Period

Daily dose (mg/kg)	0 (Control)		0.1		0.2	0.15 [a]	0.3 [b]	0.2
Number of animals (main and recovery)	M:5	F:5	M:5	F:5	M:5	F:5	M:5	F:5
Toxicokinetics								
C_{max} (ng/mL)								
Day 1	N/A	N/A	161	90.9	220	236	397	293
Day 11	N/A	N/A	159	NC [c]	311 [d]	NC [c]	N/A	345 [e]
AUC_{24} (ng·hr/mL)								
Day 1	N/A	N/A	492	387	753	642	1590	1120
Day 11	N/A	N/A	678	NC [c]	1460 [d]	NC [c]	N/A	1910 [e]
Pharmacodynamics								
E_{max} (I%)								
Day 1	N/A	N/A	61.1	51.8	67.1	55.0	79.6	69.9
Day 11	N/A	N/A	61.6	NC [c]	64.4 [d]	NC [c]	N/A	57.7

2.6.7.7F Repeat-Dose Toxicity: MLN2238: A Repeat-Dose Toxicity Study of MLN2238 Administered via Oral Gavage in the Beagle Dog With a 14-Day Recovery Period (continued)

MLN2238: A Repeat-Dose Toxicity Study of MLN2238 Administered via Oral Gavage in the Beagle Dog With a 14-Day Recovery Period										
Daily dose (mg/kg)	0 (Control)		0.1		0.2		0.15 [a]	0.3 [b]		0.2
Number of animals (main and recovery)	M:5	F:5	M:5	F:5	M:5		F:5	M:5		F:5
AUE_{24} (hr·I%)										
Day 1	N/A	N/A	801	550	1090		772	1370		1090
Day 11	N/A	N/A	779	NC [c]	907 [d]		NC [c]	N/A		932
Noteworthy findings										
Died or euthanized moribund	–	–	–	–	1 [f]		–	2 [f]		–
Clinical observations [g]					M:1 (ED)	M:4 (MS)		M:2 (ED)	M:3 (MS)	
Dehydration	–	–	–	–	+	+	–	+	+	–
Lethargy	–	–	–	–	–	+ [h]	–	+	+	–
Mucoid or soft feces	+	+	–	–	+	+	+	+	+	+
Vomiting	–	+	–	–	+	+	+	+	+	+
Physical examinations [g]										
Ataxia (weakness)	–	–	–	–	–	–	–	+	+	+
Cardiac arrhythmia	–	–	–	–	–	–	–	–	+	–
Dehydration	–	–	–	–	–	–	–	+	+	–
Ear papules	–	–	–	–	–	–	–	+	+	–
Lethargy	–	–	–	–	–	–	–	+	+	–
Tacky mucous membranes	–	–	–	–	–	–	–	+	+	–
Yellow diarrhea	–	–	–	–	–	–	–	+	+	–
Neurologic examinations [g]										
Ataxia (weakness)	–	–	–	–	–	+	+ [h]	+	+	+
Lameness	–	–	–	+	–	–	+	–	–	–
Reduced withdrawal reflex	–	–	–	–	–	–	–	–	+	–
Wide-based gait (hindlimbs)	–	–	–	–	–	–	+	–	+	–
Wide-based stance	–	–	–	–	–	–	–	+	–	–

2.6.7.7F Repeat-Dose Toxicity: MLN2238: A Repeat-Dose Toxicity Study of MLN2238 Administered via Oral Gavage in the Beagle Dog With a 14-Day Recovery Period (continued)

MLN2238: A Repeat-Dose Toxicity Study of MLN2238 Administered via Oral Gavage in the Beagle Dog With a 14-Day Recovery Period										
Daily dose (mg/kg)	0 (Control)		0.1		0.2		0.15 [a]	0.3 [b]		0.2
Number of animals (main and recovery)	M:5	F:5	M:5	F:5	M:5		F:5	M:5		F: 5
Hematology [i]										
MONO, abs (10^3/μL)	0.648	0.432	–	–	+72.1*		–	–		+85.2*
NEUT, abs (10^3/μL)	6.802	6.124	–	–	+107		–	–		+60.8*
PLT (10^3/μL)	355.2	–	–	–	–		–	+33.6*		–
WBC (10^3/μL)	10.78	9.50	–	–	+66.8		–	–		+45.3*
Coagulation [i]										
FIB (mg/dL)	208.2	164.6	–	–	+101.4*		–	–		+95.3**
Clinical chemistry [i]										
BUN (mg/dL)	14.8	–	–	–	–45.9*		–	–		–
PHOS (mg/dL)	6.28	5.88	–	–	–30.3*		–	–		–23.5*
Primary Necropsy (Day 12)										
Number of animals (main study + early death)	M:3	F:3	M:3	F:3	M:1 (ED)	M:2 (MS)	F:3	M:2 (ED)	M:0 (MS)	F:3
Organ weights [i, j]										
Thymus	9.10	9.48	–40.0	–	–60.2		–	N/A		–28.3
Macroscopic findings [k]										
Intestine [l]										
Discoloration, red or dark, mucosa	–	1	2	–	1	1	–	2	N/A	–
Microscopic findings [k]										
Dorsal root ganglion (cervical)										
Degeneration, neuronal	0	0	1	0	1	2	0	2	N/A	3
Minimal	–	–	1	–	–	1	–	–	N/A	3
Mild	–	–	–	–	–	1	–	–	N/A	–
Moderate	–	–	–	–	1	–	–	2	N/A	–

2.6.7.7F Repeat-Dose Toxicity: MLN2238: A Repeat-Dose Toxicity Study of MLN2238 Administered via Oral Gavage in the Beagle Dog With a 14-Day Recovery Period (continued)

MLN2238: A Repeat-Dose Toxicity Study of MLN2238 Administered via Oral Gavage in the Beagle Dog With a 14-Day Recovery Period										
Daily dose (mg/kg)	0 (Control)		0.1		0.2		0.15 [a]	0.3 [b]		0.2
Number of animals (main study + early death)	M:3	F:3	M:3	F:3	M:1 (ED)	M:2 (MS)	F:3	M:2 (ED)	M:0 (MS)	F:3
Microscopic findings [k] (continued)										
Dorsal root ganglion (lumbar)										
Degeneration, neuronal	0	0	1	0	1	1	0	2	N/A	1
Minimal	–	–	1	–	–	1	–	–	N/A	1
Moderate	–	–	–	–	1	–	–	2	N/A	–
Intestine [m]										
Congestion, mucosa	0	1	0	1	1	1	0	1	N/A	0
Minimal	–	1	–	1	–	1	–	–	N/A	–
Mild	–	–	–	–	1	–	–	1	N/A	–
Congestion, submucosa	0	0	0	0	0	1	0	0	N/A	0
Minimal	–	–	–	–	–	1	–	–	N/A	–
Degeneration, neuronal, end organ ganglia	0	0	0	1	1	1	1	2	N/A	1
Minimal	–	–	–	1	–	1	1	1	N/A	1
Mild	–	–	–	–	1	–	–	1	N/A	–
Edema	0	0	0	0	0	0	0	2	N/A	0
Mild	–	–	–	–	–	–	–	1	N/A	–
Moderate	–	–	–	–	–	–	–	1	N/A	–
Enteropathy	0	0	0	0	0	0	0	2	N/A	0
Minimal	–	–	–	–	–	–	–	1	N/A	–
Mild	–	–	–	–	–	–	–	1	N/A	–
Hemorrhage, mucosa	0	0	0	0	1	1	0	2	N/A	0
Minimal	–	–	–	–	–	1	–	–	N/A	–
Mild	–	–	–	–	1	–	–	2	N/A	–
Inflammation, acute, mucosa and submucosa	0	0	0	0	1	1	0	2	N/A	0
Minimal	–	–	–	–	1	–	–	1	N/A	–
Mild	–	–	–	–	–	1	–	1	N/A	–
Inflammation, chronic, active	0	0	0	0	0	1	0	0	N/A	0
Minimal	–	–	–	–	–	1	–	–	N/A	–

MLN2238: A Repeat-Dose Toxicity Study of MLN2238 Administered via Oral Gavage in the Beagle Dog With a 14-Day Recovery Period										
Daily dose (mg/kg)	0 (Control)		0.1		0.2		0.15 [a]	0.3 [b]		0.2
Inflammation, subacute, mucosa, submucosa, and perivascular	1	1	0	0	1	2	0	2	N/A	1
Minimal	1	1	–	–	1	2	–	1	N/A	1
Mild	–	–	–	–	–	–	–	1	N/A	–

2.6.7.7F Repeat-Dose Toxicity: MLN2238: A Repeat-Dose Toxicity Study of MLN2238 Administered via Oral Gavage in the Beagle Dog With a 14-Day Recovery Period (continued)

MLN2238: A Repeat-Dose Toxicity Study of MLN2238 Administered via Oral Gavage in the Beagle Dog With a 14-Day Recovery Period										
Daily dose (mg/kg)	0 (Control)		0.1		0.2		0.15 [a]	0.3 [b]		0.2
Number of animals (main study + early death)	M:3	F:3	M:3	F:3	M:1 (ED)	M:2 (MS)	F:3	M:2 (ED)	M:0 (MS)	F:3
Microscopic findings [k] (continued)										
Lymph nodes [n]										
Lymphoid depletion	0	0	0	0	1	0	0	1	N/A	0
Minimal	–	–	–	–	1	–	–	–	N/A	–
Mild	–	–	–	–	–	–	–	1	N/A	–
Peyer's patch										
Depletion, lymphoid	0	0	0	0	0	0	0	0	N/A	1
Minimal	–	–	–	–	–	–	–	–	N/A	1
Necrosis	0	0	0	0	1	0	0	2	N/A	0
Moderate	–	–	–	–	1	–	–	–	N/A	–
Marked	–	–	–	–	–	–	–	2	N/A	–
Salivary gland, mandibular										
Degeneration, neuronal, end organ ganglia	0	0	0	0	1	1	0	1	N/A	0
Minimal	–	–	–	–	–	1	–	1	N/A	–
Mild	–	–	–	–	1	–	–	–	N/A	–
Spinal cord, thoracic										
Degeneration, axonal, dorsal column	0	0	0	0	1	0	1	0	N/A	0
Minimal	–	–	–	–	1	–	1	–	N/A	–
Spleen										
Infiltration, neutrophilic	0	0	0	0	1	2	0	2	N/A	0
Minimal	–	–	–	–	1	2	–	1	N/A	–
Mild	–	–	–	–	–	–	–	1	N/A	–

2.6.7.7F Repeat-Dose Toxicity: MLN2238: A Repeat-Dose Toxicity Study of MLN2238 Administered via Oral Gavage in the Beagle Dog With a 14-Day Recovery Period (continued)

MLN2238: A Repeat-Dose Toxicity Study of MLN2238 Administered via Oral Gavage in the Beagle Dog With a 14-Day Recovery Period										
Daily dose (mg/kg)	0 (Control)		0.1		0.2		0.15 [a]	0.3 [b]		0.2
Number of animals (main study + early death)	M:3	F:3	M:3	F:3	M:1 (ED)	M:2 (MS)	F:3	M:2 (ED)	M:0 (MS)	F:3
Microscopic findings [k] (continued)										
Stomach										
Degeneration, neuronal, end organ ganglia	0	0	0	0	1	1	0	1	N/A	0
Minimal	–	–	–	–	–	1	–	–	N/A	–
Mild	–	–	–	–	1	–	–	1	N/A	–
Depletion, lymphoid, mucosa-associated lymphoid tissue	0	0	0	0	0	0	0	2	N/A	0
Minimal	–	–	–	–	–	–	–	1	N/A	–
Moderate	–	–	–	–	–	–	–	1	N/A	–
Inflammation, acute, transmural	0	0	0	0	1	0	0	0	N/A	0
Moderate	–	–	–	–	1	–	–	–	N/A	–
Inflammation, chronic, pylorus	0	0	0	0	1	0	0	0	N/A	0
Minimal	–	–	–	–	1	–	–	–	N/A	–
Ulceration, pylorus	0	0	0	0	1	0	0	0	N/A	0
Mild	–	–	–	–	1	–	–	–	N/A	–
Sympathetic ganglion (abdominal and thoracic)										
Degeneration, neuronal	0	0	0	0	1	2	0	2	N/A	1
Minimal	–	–	–	–	–	2	–	–	N/A	1
Moderate	–	–	–	–	1	–	–	2	N/A	–
Thymus										
Depletion, cellular, cortex	0	0	0	0	1	2	0	2	N/A	0
Minimal	–	–	–	–	–	2	–	–	N/A	–
Mild	–	–	–	–	–	–	–	1	N/A	–
Moderate	–	–	–	–	1	–	–	1	N/A	–

2.6.7.7F Repeat-Dose Toxicity: MLN2238: A Repeat-Dose Toxicity Study of MLN2238 Administered via Oral Gavage in the Beagle Dog With a 14-Day Recovery Period (continued)

MLN2238: A Repeat-Dose Toxicity Study of MLN2238 Administered via Oral Gavage in the Beagle Dog With a 14-Day Recovery Period								
Daily dose (mg/kg)	0 (Control)		0.1		0.2	0.15 [a]	0.3 [b]	0.2
Recovery period evaluations								
Number of animals (recovery)	M:2	F: 2	M:2	F: 2	M:2	F: 2	M:3	F: 2
Noteworthy findings								
Neurologic examinations [g]								
Ataxia (weakness)	–	–	–	–	+	–	+	+
Reduced extensor carpi reflex	–	–	–	–	+	–	+	–
Wide-based stance	–	–	–	–	+	–	+	–
Recovery Necropsy (Day 26)								
Microscopic findings [k]								
Brain								
Degeneration, axonal, trigeminal tract	0	0	0	0	2	0	1	2
Minimal	–	–	–	–	2	–	1	2
Dorsal root ganglion (cervical)								
Degeneration, neuronal	0	0	0	0	1	0	2	2
Minimal	–	–	–	–	1	–	2	2
Degeneration, nerve fiber	0	0	0	0	2	0	3	2
Minimal	–	–	–	–	1	–	3	2
Mild	–	–	–	–	1	–	–	–
Dorsal root ganglion (lumbar)								
Degeneration, neuronal	0	0	0	0	2	0	1	1
Minimal	–	–	–	–	2	–	1	1
Degeneration, nerve fiber	0	0	0	0	2	0	2	2
Minimal	–	–	–	–	2	–	2	–
Mild	–	–	–	–	–	–	–	1
Moderate	–	–	–	–	–	–	–	1
Intestine [o]								
Congestion, mucosa	0	0	0	0	0	0	1	0
Minimal	–	0	–	–	–	–	1	–

2.6.7.7F Repeat-Dose Toxicity: MLN2238: A Repeat-Dose Toxicity Study of MLN2238 Administered via Oral Gavage in the Beagle Dog With a 14-Day Recovery Period (continued)

MLN2238: A Repeat-Dose Toxicity Study of MLN2238 Administered via Oral Gavage in the Beagle Dog With a 14-Day Recovery Period

Daily dose (mg/kg)	0 (Control)		0.1		0.2	0.15 [a]	0.3 [b]	0.2
Number of animals (recovery)	M:2	F: 2	M:2	F: 2	M:2	F: 2	M:3	F: 2
Enteropathy	0	0	0	0	0	0	2	0
Minimal	–	–	–	–	–	–	2	–
Inflammation, chronic, mucosa	0	0	0	0	1	0	1	0
Minimal	–	–	–	–	1	–	1	–
Sciatic nerve								
Degeneration	0	0	0	0	2	0	3	2
Mild	–	–	–	–	2	–	3	1
Moderate	–	–	–	–	–	–	–	1
Spinal cord [p]								
Degeneration, axonal, dorsal column	0	0	0	0	2	0	3	2
Minimal	–	–	–	–	2	–	2	1
Mild	–	–	–	–	–	–	1	1
Sural nerve								
Degeneration	0	0	0	1	1	0	1	2
Minimal	–	–	–	1	1	–	1	–
Mild	–	–	–	–	–	–	–	1
Moderate	–	–	–	–	–	–	–	1
Sympathetic ganglion (abdominal)								
Degeneration, neuronal	0	0	0	0	1	0	1	0
Minimal	–	–	–	–	1	–	1	–
Thymus								
Depletion, cellular, cortex	0	0	0	0	1	0	2	0
Minimal	–	–	–	–	1	–	1	–
Moderate	–	–	–	–	–	–	1	–

* $p \leq 0.05$ (Dunnett test); ** $p \leq 0.05$ (Kruskal-Wallis with Dunn procedure); - = no noteworthy findings; + = finding present; abs = absolute; AUC_{24} = area under the plasma concentration-time curve from the time 0 to 24 hours; AUE_{24} = area under the effect-time curve from the time 0 to 24 hours; BIW = twice weekly; BUN = blood urea nitrogen; C_{max} = maximum observed plasma concentration; ED = early death; Emax = maximum effect; F = female(s); FIB = fibrinogen; GLP = Good Laboratory Practice; HNSTD = highest nonseverely toxic dose; I% = percentage of proteasome inhibition; M = male(s); MONO = monocyte; MS = main study; N/A = not applicable; NC = not calculated;

NEUT = neutrophil; NR = not reported; PHOS = phosphorus; PLT = platelet; WBC = white blood cell; w:w = weight-to-weight ratio.

Note: A separate, non-GLP-compliant analysis was performed that determined that dose-limiting toxicity (DLT) was associated with plasma exposure and blood AUE_{24} (Report4.2.3.2-12).

a Because of the toxicity seen in males dosed at 0.3 mg/kg, the doses for females were adjusted to 0.1, 0.15, and 0.2 mg/kg.

b Due to toxicity seen in the high-dose males (0.3 mg/kg), dosing was discontinued for this group after Day 1, and animals were instead sent to necropsy on Day 26 as recovery animals.
c Not calculated due to the outlier toxicokinetic (TK) and pharmacodynamic (PD) profile for this group of animals.
d n=4; 1 male was euthanized in extremis on Day 9.
e n=3; TK data from 2 animals were determined to be outliers and were excluded from mean TK parameter calculations.
f Deaths were test article-related. The 0.2- and 0.3 mg/kg males were euthanized on Days 9 and 5, respectively, following clinical signs of gastrointestinal (GI) disturbances and dehydration, accompanied by decreased food consumption and body weight loss.
g +/- indicates presence or absence of findings.
h Finding only occurred in 1 animal and was transient in nature, and therefore, was not considered test article-related for this dose group.
i At the end of the dosing period (Day 12). For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not percent differences).
j Both absolute and relative weights differed from controls in the direction indicated. Number indicates percent difference for the absolute organ weights. Organ weights were not recorded for early death animals.
k Number represents number of animals with finding present.
l Includes findings in the cecum and ileum.
m Includes all sections of the intestine. When severity of findings differed amongst sites, the highest level of severity is presented.
n Includes findings in mandibular and mesenteric lymph nodes. When severity of findings differed amongst sites, the highest level of severity is presented.
o Includes findings in cecum and rectum. When severity of findings differed amongst sites, the highest level of severity is presented.
p Includes findings in cervical, lumbar, and thoracic portions of spine. When severity of findings differed amongst sites, the highest level of severity is presented .

2.6.7.7G Repeat-Dose Toxicity: A 5 Cycle Oral (Gavage) Toxicity Study of MLN9708 in Beagle Dogs With a 14-Day Recovery Period

Species/strain:	Dog/beagle	Duration of dosing:	94 days (5 cycles of BIW dosing for 2 consecutive weeks separated by a 10-day nondosing observation period)	Test article:	Ixazomib citrate
Initial age:	Approximately 6 months	Duration of recovery period:	14 days	Report:	4.2.3.2-13
Date of first dose:	[redacted] (Males) [redacted] (Females)	Day of first dose (Day 0 or Day 1):	Day 0	GLP compliance:	Yes
Method of administration:	Oral gavage				
Vehicle/formulation:	[redacted] and 0.45% NaCl in Sterile Water For Injection (SWI), USP [redacted]				
Special features:	Pharmacodynamic analysis (20S proteasome activity, assessed by fluorometry) and blood pharmacokinetic (PK) analysis were done in a separate, non-GLP-compliant study (Report 4.2.3.2-14).				
Study endpoint:	MTD = 0.15 mg/kg; NOEL = 0.05 mg/kg				

A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in Beagle Dogs With a 14-Day Recovery Period								
Daily dose (mg/kg)[a]	0 (Control)		0.05		0.10		0.15	
Number of animals (main and recovery)	M:6	F:6	M:3	F:3	M:6	F:6	M:6	F:6
Plasma toxicokinetics[b]								
C_{max} (ng/mL)								
Day 0	N/A		27.7		53.2		82.0	
Day 94	N/A		43.5		80.5		137	
AUC_{24} (ng·hr/mL)								
Day 0	N/A		155		307		495	
Day 94	N/A		388		750		1260	

2.6.7.7G Repeat-Dose Toxicity: A 5 Cycle Oral (Gavage) Toxicity Study of MLN9708 in Beagle Dogs With a 14-Day Recovery Period (continued)

A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in Beagle Dogs With a 14-Day Recovery Period								
Daily dose (mg/kg)[a]	0 (Control)		0.05		0.10		0.15	
Number of animals (main and recovery)	M:6	F:6	M:3	F:3	M:6	F:6	M:6	F:6
Pharmacodynamics[b]								
E_{max} (I%)								
Day 0	N/A		20.1		34.2		34.5	
Day 94	N/A		30.6		35.7		46.8	
AUE_{24} (hr·I%)								
Day 0	N/A		150		346		356	
Day 94	N/A		330		443		581	
Whole Blood pharmacokinetics[b]								
C_{max} (ng/mL)								
Day 0	N/A		65.9		128		188	
Day 94	N/A		139		214		294	
AUC_{24} (ng·hr/mL)								
Day 0	N/A		1240		2370		3200	
Day 94	N/A		2690		3890		4890	
Noteworthy findings								
Died or euthanized moribund	–	1[c]	–	–	–	–	–	–
Primary Necropsy (Day 95)								
Number of animals (main study)	M:3	F:3	M:3	F:3	M:3	F:3	M:3	F:3
Microscopic findings[d]								
Dorsal root ganglion								
Degeneration, nerve fiber	0	1	0	0	1	0	0	2
Minimal	–	1	–	–	1	–	–	1
Mild	–	–	–	–	–	–	–	1
Degeneration, neuronal	0	0	0	0	0	0	1	1
Minimal	–	–	–	–	–	–	1	1

2.6.7.7G Repeat-Dose Toxicity: A 5 Cycle Oral (Gavage) Toxicity Study of MLN9708 in Beagle Dogs With a 14-Day Recovery Period (continued)

A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in Beagle Dogs With a 14-Day Recovery Period								
Daily dose (mg/kg)[a]	0 (Control)		0.05		0.10		0.15	
Number of animals (main study)	M:3	F:3	M:3	F:3	M:3	F:3	M:3	F:3
Microscopic findings[d] (continued)								
Peripheral autonomic ganglion (salivary gland)								
Degeneration, neuronal	0	0	0	0	0	0	1	2
Minimal	–	–	–	–	–	–	1	2
Sciatic nerve								
Degeneration	0	0	0	1	1	2	3	3
Minimal	–	–	–	1	1	2	3	3
Spinal cord								
Degeneration, axonal, dorsal columns	0	0	0	0	0	1	2	2
Minimal	–	–	–	–	–	1	2	2
Sural nerve								
Degeneration	0	0	0	0	0	1	0	2
Minimal	–	–	–	–	–	1	–	2
Sympathetic ganglion								
Degeneration, neuronal	0	0	0	0	1	1	2	3
Minimal	–	–	–	–	1	1	2	1
Mild	–	–	–	–	–	–	–	2
Vagus nerve								
Degeneration	0	0	0	0	1	0	0	2
Minimal	–	–	–	–	1	–	–	2

2.6.7.7G Repeat-Dose Toxicity: A 5 Cycle Oral (Gavage) Toxicity Study of MLN9708 in Beagle Dogs With a 14-Day Recovery Period (continued)

A 5-Cycle Oral (Gavage) Toxicity Study of MLN9708 in Beagle Dogs With a 14-Day Recovery Period								
Daily dose (mg/kg)[a]	0 (Control)		0.05		0.10		0.15	
Recovery evaluations								
Number of animals (recovery)	M:3	F:3	M: 0	F: 0	M:3	F:3	M:3	F:3
Recovery Necropsy (Day 109)								
Microscopic findings[d]								
Dorsal root ganglion								
Degeneration, nerve fiber	1	1	N/A	N/A	1	0	1	1
Minimal	1	1	N/A	N/A	1	–	1	–
Mild	–	–	N/A	N/A	–	–	–	1
Degeneration, neuronal	0	0	N/A	N/A	0	0	0	1
Minimal	–	–	N/A	N/A	–	–	–	1
Sciatic nerve								
Degeneration	0	1	N/A	N/A	0	0	0	1
Minimal	–	1	N/A	N/A	–	–	–	1
Spinal cord								
Degeneration, axonal, dorsal columns	0	0	N/A	N/A	0	0	1	1
Minimal	–	–	N/A	N/A	–	–	1	1
Sural nerve								
Degeneration	0	1	N/A	N/A	0	0	1	1
Minimal	–	1	N/A	N/A	–	–	1	1
Sympathetic ganglion								
Degeneration, nerve fiber	0	0	N/A	N/A	0	0	0	1
Minimal	–	–	N/A	N/A	–	–	–	1
Vagus nerve								
Degeneration	0	0	N/A	N/A	0	1	0	1
Minimal	–	–	N/A	N/A	–	1	–	1

– = no noteworthy findings; AUC_{24} = area under the plasma concentration-time curve from the time 0 to 24 hours; AUE_{24} = area under the effect-time curve from the time 0 to 24 hours; BIW = twice weekly; C_{max} = maximum observed plasma concentration; Emax = maximum effect; F = female(s); GLP = Good Laboratory Practice; I% = percentage of proteasome inhibition; M = male(s); MTD = maximum tolerated dose; N/A = not applicable; NOEL = no observed effect level;
PK = pharmacokinetic(s); USP = United States Pharmacopeia.

a Doses are presented as mg/kg ixazomib.

b On the basis of the whole blood PK data, there were no pronounced sex-related differences in exposure to ixazomib, and exposure to ixazomib increased as the dose increased. A similar conclusion was drawn from the plasma toxicokinetic (TK) data; see Tabulated Summary 2.6.7.3.

c Death was not test article-related. Animal was euthanized on Day 105 after being found out of cage and with sperm detected in a vaginal smear.

d Number represents number of animals with finding present in tissues examined.

2.6.7.7H Repeat-Dose Toxicity: A 9-Month Oral Gavage Toxicity Study of MLN9708 With a 2-Week Recovery Period in Beagle Dogs

Species/strain:	Dog/beagle	Duration of dosing:	266 days (10 cycles of QW × 3 dosing separated by a 13-day nondosing period)	Test article:	Ixazomib citrate
Initial age:	12 - 14 months	Duration of recovery period:	14 days	Report:	4.2.3.2-15
Date of first dose:	[redacted]	Day of first dose (Day 0 or Day 1):	Day 0	GLP compliance:	Yes
Method of administration:	Oral gavage				
Vehicle/formulation:	[redacted] and 0.45% NaCl, in Sterile Water For Injection (SWI), USP [redacted]				
Special features:	FOB, ECG				
Study endpoint:	NOAEL = 0.1 mg/kg				

A 10-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Beagle Dog With a 14-Day Recovery Period

Daily dose (mg/kg)[a]	0 (Control)		0.05		0.10		0.20	
Number of animals (main and recovery):	M:6	F:6	M:3	F:3	M:6	F:6	M:6	F:6
Toxicokinetics[b]								
C_{max}(ng/mL)								
Day 0	N/A		38.9		107		277	
Day 14	N/A		36.2		97.5		217	
Day 252	N/A		57.1		136		333	
Day 266	N/A		83.5		162		353	
AUC_{24} (ng·hr/mL)								
Day 0	N/A		218		609[c]		1410	
Day 14	N/A		206[d]		638[e]		1390	
Day 252	N/A		309		695		1560	
Day 266	N/A		410		828		1870	

2.6.7.7H Repeat-Dose Toxicity: A 9-Month Oral Gavage Toxicity Study of MLN9708 With a 2-Week Recovery Period in Beagle Dogs (continued)

A 10-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Beagle Dog With a 14-Day Recovery Period								
Daily dose (mg/kg)[a]	0 (Control)		0.05		0.10		0.20	
Number of animals (Main and recovery):	M:6	F:6	M:3	F:3	M:6	F:6	M:6	F:6
Toxicokinetics[b] (continued)								
AUC_{168} (ng·hr/mL)								
Day 0	N/A		706		1900		3300	
Day 14	N/A		1070		2610		3250	
Day 252	N/A		934		1940		3900	
Day 266[f]	N/A		N/A		N/A		N/A	
Noteworthy findings								
Died or euthanized moribund	–	–	–	–	–	–	–	–
Dosing period evaluations								
ECG	–	–	–	–	–	–	–	–
FOB	–	–	–	–	–	–	–	–
Hematology[g, h]								
ABS LYMPH (10^3/μL)	–	2.51	–	–	–	–	–	–26
ABS MONO (10^3/μL)	0.55	0.37	–	–	–	–	87	127**
ABS NEUT (10^3/μL)	6.12	4.90	–	–	–	–	62	65**
ABS WBC (10^3/μL)	9.56	8.11	–	–	–	–	40	37*
Clinical chemistry[g, h]								
AST (U/L)	31	–	–	–	–	–	36**	–
Phosphorus (mg/dL)	4.7	4.6	–	–	–	–	–19	–20*

2.6.7.7H Repeat-Dose Toxicity: A 9-Month Oral Gavage Toxicity Study of MLN9708 With a 2-Week Recovery Period in Beagle Dogs (continued)

A 10-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Beagle Dog With a 14-Day Recovery Period								
Daily dose (mg/kg)[a]	0 (Control)		0.05		0.10		0.20	
Primary necropsy (Day 267)								
Number of Animals (Main Study)	M:3	F:3	M:3	F:3	M:3	F:3	M:3	F:3
Macroscopic findings								
Stomach								
Area(s), dark red	0	0	0	0	0	0	0	1
Microscopic findings[i]								
Brain								
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
Dorsal root ganglion, lumbar								
Degeneration, nerve fiber	0	0	0	1	0	0	1	2
Minimal	–	–	–	1	–	–	1	2
Degeneration, neuronal	0	0	0	0	0	0	3	2
Minimal	–	–	–	–	–	–	3	2
Intestine[j]								
Infiltrate, neutrophil	0	0	2	0	0	1	1	2
Minimal	–	–	2	–	–	1	1	2
Lymph node, mesenteric								
Depletion, lymphoid	0	0	0	0	0	0	1	0
Minimal	–	–	–	–	–	–	1	–
Infiltrate, neutrophil	0	0	0	0	0	0	1	1
Minimal	–	–	–	–	–	–	1	–
Mild	–	–	–	–	–	–	–	1

2.6.7.7H Repeat-Dose Toxicity: A 9-Month Oral Gavage Toxicity Study of MLN9708 With a 2-Week Recovery Period in Beagle Dogs (continued)

A 10-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Beagle Dog With a 14-Day Recovery Period								
Daily dose (mg/kg)[a]	0 (Control)		0.05		0.10		0.20	
Number of Animals (Main Study)	M:3	F:3	M:3	F:3	M:3	F:3	M:3	F:3
Microscopic findings[i] (continued)								
Nerve, sciatic								
Degeneration, nerve fiber	0	0	1	0	0	0	3	3
Minimal	–	–	1	–	–	–	3	3
Nerve, vagus								
Degeneration, nerve fiber	0	0	0	0	0	0	1	1
Minimal	–	–	–	–	–	–	1	1
Peyer's patches								
Depletion, lymphoid	0	0	0	0	0	0	0	1
Mild	–	–	–	–	–	–	–	1
Infiltrate, neutrophil	0	0	0	0	0	0	0	1
Mild	–	–	–	–	–	–	–	1
Salivary gland, mandibular								
Degeneration, neuronal	0	0	0	0	0	0	3	2
Minimal	–	–	–	–	–	–	3	2
Spinal cord[k]								
Degeneration, axonal, dorsal columns	0	0	0	1	0	0	3	3
Minimal	–	–	–	1	–	–	3	3
Gliosis, dorsal column	0	0	0	0	0	0	1	1
Minimal	–	–	–	–	–	–	1	1
Stomach								
Erosion	0	0	0	0	0	0	1	0
Minimal	–	–	–	–	–	–	1	–
Infiltrate, neutrophil	0	0	0	0	1	1	1	2
Minimal	–	–	–	–	1	1	1	2

2.6.7.7H Repeat-Dose Toxicity: A 9-Month Oral Gavage Toxicity Study of MLN9708 With a 2-Week Recovery Period in Beagle Dogs (continued)

A 10-Cycle Oral (Gavage) Toxicity Study of MLN9708 in the Beagle Dog With a 14-Day Recovery Period								
Daily dose (mg/kg)[a]	0 (Control)		0.05		0.10		0.20	
Number of Animals (Main Study)	M:3	F:_3	M:3	F:3	M:3	F:3	M:3	F:3
Microscopic findings[i] (continued)								
Sympathetic ganglion, celiac								
Degeneration, neuronal	0	0	0[l]	0	0	0	3	3
Minimal	–	–	–	–	–	–	3	3
Sympathetic ganglion, stellate								
Degeneration, nerve fiber	0	0	0	0	0	0	2[l]	0
Minimal	–	–	–	–	–	–	2	–
Degeneration, neuronal	0	0	0	0	0	1	1[l]	3
Minimal	–	–	–	–	–	1	1	3
Recovery evaluations								
Number of animals (recovery):	M:3	F:3	M:0	F:0	M:3	F:3	M:3	F:3
Recovery necropsy (Day 280)								
Microscopic findings[i]								
Dorsal root ganglion, lumbar								
Degeneration, nerve fiber	0	0	N/A	N/A	0	0	3	1
Minimal	–	–	N/A	N/A	–	–	3	1
Ileum								
Infiltrate, neutrophil	0	0	N/A	N/A	0	1	0	1
Minimal	–	–	N/A	N/A	–	1	–	1
Spinal cord								
Degeneration, axonal, dorsal columns	0	1	N/A	N/A	0	0	1	2
Minimal	–	1	N/A	N/A	–	–	1	2

*p < 0.05, **p < 0.01 (Dunnett test); – = no noteworthy findings; ABS LYMPH = absolute lymphocytes; ABS MONO = absolute monocytes; ABS NEUT = absolute neutrophils; ABS WBC = absolute white blood cells; AST = aspartate aminotransferase; AUC_{24} = area under the plasma concentration-time curve from the time 0 to 24 hours; AUC_{168} = area under the plasma concentration-time curve from the time 0 to 168 hours; C_{max} = maximum observed plasma concentration; ECG = electrocardiogram; F = female(s); FOB = functional observational battery; GLP = Good Laboratory Practice; M = male(s); N/A = not applicable; NOAEL = no observed adverse effect level; QW = once weekly; USP = United States Pharmacopeia.

a Doses are presented as mg/kg ixazomib.

b Due to lack of sex differences, toxicokinetic (TK) data is presented as sexes combined.

c n = 9.

d n = 4.

e n = 7.

f TK samples only collected up to 24 hours on Day 266.

g At the end of the dosing period (Week 38).

h For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not percent differences).

i Numbers indicated total incidence observed in tissues examined.

j Includes lesions found in the duodenum, ileum, and rectum. When findings of differing severities were found in different parts of the intestine, the highest severity is presented.

k Tissue not examined < 0.2 mg/kg, with the exception of 1 0.1-mg/kg female.

l Tissue was not present from 1 animal during examination, and therefore was examined from only 2 animals.

2.6.7.7I Repeat-Dose Toxicity: A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs

Species/strain:	Dog/beagle	Duration of dosing:	32 days (2 cycles of BIW dosing for 2 weeks, separated by a 10-day nondosing period)	Test article:	Ixazomib
Initial age:	6 - 7 months	Duration of postdose:	14 days (following dosing Cycle 2)	Report:	4.2.3.2-17
Date of first dose:	[redacted] (males) [redacted] (females)	Day of first dose (Day 0 or Day 1):	Day 0	GLP compliance:	Yes
Method of administration:	5-minute IV infusion				
Vehicle/formulation:	[redacted] 1% propylene glycol, [redacted]				
Special features:	Pharmacodynamic analysis (20S proteasome activity, assessed by fluorometry) was done in a separate, non-GLP-compliant study (Report : 4.2.3.2-18).				
Study endpoint:	HNSTD = 0.14 mg/kg				

A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs

Daily dose (mg/kg)[a]	0 (Control)		0.10		0.14		0.18		0.25	
Number of animals (main and recovery)	M:6(5) [b]	F:6(5) [b]	M:6	F:6	M:3	F:3	M:6	F:6	M:6	F:6
Toxicokinetics										
Plasma C_{max} (ng/mL) [c]										
Day 0	N/A		362		N/A		813		856	
Day 10	N/A		465		N/A		1060 [d]		N/A	
Day 21	N/A		312		452		N/A		N/A	
Day 31	N/A		368		636		N/A		N/A	

2.6.7.7I Repeat-Dose Toxicity: A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs (continued)

A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs										
Daily dose (mg/kg)[a]	0 (Control)		0.10		0.14		0.18		0.25	
Number of animals (main and recovery)	M:6(5) [b]	F:6(5) [b]	M:6	F:6	M:3	F:3	M:6	F:6	M:6	F:6
Toxicokinetics (continued)										
Plasma AUC_{24} (ng·hr/mL) [c]										
Day 0	N/A		512		N/A		1080		1500	
Day 10	N/A		1100		N/A		2680 [d]		N/A	
Day 21	N/A		518		547		N/A		N/A	
Day 31	N/A		1240		1550		N/A		N/A	
Pharmacodynamics										
E_{max} (I%) [c]										
Day 0	6.83		56.7		N/A		76.3		74.1	
Day 10	N/A		64.7		N/A		61.9 [d]		N/A	
Day 21	N/A		72.7		73.4		N/A		N/A	
Day 31	N/A		79.5		74.7		N/A		N/A	
AUE_{24} (hr·I%) [c]										
Day 0	70.4		420		N/A		821		1020	
Day 10	N/A		537		N/A		851 [d]		N/A	
Day 21	N/A		486		588		N/A		N/A	
Day 31	N/A		679		843		N/A		N/A	

2.6.7.7I Repeat-Dose Toxicity: A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs (continued)

A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs														
Daily dose (mg/kg)[a]	0 (Control)		0.10		0.14		0.18				0.25			
Number of animals (main and recovery)	M:6(5)[b]	F:6(5)[b]	M:6	F:6	M:3	F:3	M:6		F:6		M:6		F:6	
Noteworthy findings														
Died or euthanized moribund	–	–	–	–	–	–	2[e, f]		1[f]		2[f]		3[e, f]	
Body weight (kg)														
Day 7	9.8	8.5	–	–	N/A	N/A	–5.1		–4.7		–11.2		–14.1	
Day 14	9.8	8.6	–	–	N/A	N/A	–8.2		–9.3		–9.2		N/A	
Food consumption														
Decrease	–		–		–		+ (0.18 mg/kg)				+ (0.25 mg/kg)			
Clinical observations[g]	M:6(5)[b]	F:6(5)[b]	M:6	F:6	M:3	F:3	M:2 (ED)	M:4 (MS)	F:1 (ED)	F:5 (MS)	M:2 (ED)	M:4 (MS)	F:3 (ED)	F:3 (MS)
Body cool to touch	–	–	–	–	–	–	+	–	–	–	+	–	+	–
Brown material anogenital area or hindlimbs	–	–	–	–	–	–	+	–	–	–	+	–	+	–
Decreased activity	–	–	–	–	–	–	+	+	+	+	+	+	–	–
Defecation decreased	–	–	–	–	–	–	–	+	–	+	+	+	+	+
Dermal atonia	–	–	–	–	–	–	+	–	–	–	+	+	+	–
Diarrhea, with or without red and/ or black material	–	+	–	+	–	+	+	+	+	+	+	+	+	+
Ears cool to touch	–	–	–	–	–	–	+	–	+	–	+	–	–	–
Emesis containing food, red, white, and/or yellow material	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Red, green, and/ or yellow mucoid feces	+	–	+	–	–	–	+	+	+	+	+	+	+	+
Feces, soft	+	+	+[h]	+[h]	+[h]	+[h]	+	+	+	+	+	+	+	+
Hypoactivity	–	–	–	–	–	–	+	–	+	–	+	+	+	+

2.6.7.7I Repeat-Dose Toxicity: A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs (continued)

A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs														
Daily dose (mg/kg)[a]	0 (Control)		0.10		0.14		0.18				0.25			
Number of animals (main and recovery)	M:6(5) [b]	F:6(5) [b]	M:6	F:6	M:3	F:3	M:6		F:6		M:6		F:6	
Clinical observations[g] (continued)	M:6(5) [b]	F:6(5) [b]	M:6	F:6	M:3	F:3	M:2 (ED)	M:4 (MS)	F:1 (ED)	F:5 (MS)	M:2 (ED)	M:4 (MS)	F:3 (ED)	F:3 (MS)
Impaired equilibrium	–	–	–	–	–	–	+	+	+	+	+	+	+	+
Impaired muscle coordination	–	–	–	–	–	–	+	+	+	+	+	+	+	+
Impaired use of limbs	–	–	–	–	–	–	+	+	+	–	+	+	+	–
Open sore urogenital area	–	–	–	–	–	–	–	–	–	–	+	–	–	–
Partial closure eye(s)	–	–	–	–	–	–	+	–	+	–	+	+	+	–
Prostrate	–	–	–	–	–	–	+	–	–	–	+	–	–	–
Red material, anogenital area or hindlimbs	–	–	–	–	–	–	+	–	–	–	–	–	+	–
Respiration labored/rate decreased	–	–	–	–	–	–	+	–	+	–	+	–	+	–
Thin	–	–	–	–	–	–	+	+	+	+	+	+	+	+
Tremors intermittent	–	–	–	–	–	–	+	–	+	–	+	–	+	–
Neurologic observations [g]														
Ataxia and/ or low to very low arousal	–	–	–	+[i]	–	+	+	+	+	+	+	+	+[i]	+[i]

2.6.7.7I Repeat-Dose Toxicity: A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs (continued)

A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs										
Daily dose (mg/kg)[a]	0 (Control)		0.10		0.14		0.18		0.25	
Number of animals (main and recovery)	M:6(5) [b]	F:6(5) [b]	M:6	F:6	M:3	F:3	M:6	F:6	M:6	F:6
Hematology and Coagulation [j]										
Day 11										
LEUK ($10^3/\mu L$)	8.30	8.91	–	–	N/A	N/A	–	–	+110.0* [i]	+19.5 [k]
NEUT ($10^3/\mu L$)	4.86	5.20	–	–	N/A	N/A	+103.5	+51.2	+154.3* [i]	+18.7 [k]
MONO ($10^3/\mu L$)	0.52	0.49	–	–	N/A	N/A	+88.5	+40.8	+192.3*	+75.5 [k]
PLT ($10^3/\mu L$)	332	377	–	–	N/A	N/A	+22.0	+1.6	+33.7* [i]	+27.1
RETIC ($10^3/\mu L$)	37.8	68.0	–	–	N/A	N/A	–41.5	–29.4	–51.1*	–51.5
FIB (mg/dL)	213.3	201.2	–	–	N/A	N/A	+156.6**	+119.0**	+106.1**	+59.9
Day 20										
PLT ($10^3/\mu L$)	348	–	–	–	–	–	+37.4*	–	N/A	N/A
Day 32										
NEUT ($10^3/\mu L$)	6.04	–	–	–	+40.9	–	–	N/A	N/A	N/A
MONO ($10^3/\mu L$)	0.65	–	–	–	+27.7	–	–	N/A	N/A	N/A
RETIC ($10^3/\mu L$)	53.8	–	–	–	–31.6	–	–	N/A	N/A	N/A
Clinical Chemistry [j]										
Day 11										
A:G	1.75	1.86	–	–	N/A	N/A	–29.7**	–25.3*	–34.9**	–28.0*
ALB (g/dL)	3.3	3.4	–	–	N/A	N/A	–3.0	–2.9	–18.2**	–5.9
ALP (U/L)	85	79	–	–	N/A	N/A	+67.1	+113.9	+117.6	+39.2
CA (mg/dL)	10.8	10.9	–	–	N/A	N/A	–5.6	+0.9	–6.5*	–0.9
CHOL (mg/dL)	151	147	–	–	N/A	N/A	+51.7**	+26.5	+27.8	+4.1
GLOB (g/dL)	1.9	1.8	–	–	N/A	N/A	+36.8**	+33.3**	+26.3*	+27.8*

2.6.7.7I Repeat-Dose Toxicity: A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs (continued)

A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs														
Daily dose (mg/kg)[a]	0 (Control)		0.10		0.14		0.18				0.25			
Number of animals (main and recovery)	M:6(5) [b]	F:6(5) [b]	M:6	F:6	M:3	F:3	M:6		F:6		M:6		F:6	
Clinical Chemistry (continued)[j]														
PHOS (mg/dL)	6.8	6.4	–	–	N/A	N/A	–17.6		–4.7		–13.2		–3.1	
TP (g/dL)	5.2	5.2	–	–	N/A	N/A	+11.5		+9.6*		–1.9		+5.8	
TRIG (mg/dL)	24	29	–	–	N/A	N/A	+125.0*		+55.2		+100.0		+37.9	
Day 20														
A:G	1.61	1.80	–	–	–	–	–11.8*		-8.3		N/A		N/A	
ALB (g/dL)	3.5	3.4	–	–	–	–	–8.6*		–2.9		N/A		N/A	
Primary Necropsy (Day 32) [l]														
Number of animals (primary necropsy and early death)	M:3	F:3	M:3	F:3	M:3	F:3	M:2 (ED)	M:0 (MS)	F:1 (ED)	F:0 (MS)	M:2 (ED)	M:4 (MS)	F:3 (ED)	F:3 (MS)
Organ Weights (g) [j, m]														
Liver	271.7	223.8	–	+16.0	–	+27.2*	N/A		N/A		N/A		N/A	
Macroscopic findings [n]														
Intestines														
Mucosa, reddened	–	1	–	–	2	1	1	N/A	1	N/A	1	1	3	–
Intussusception	–	–	–	–	–	–	1	N/A	–	N/A	2	–	1	–
Lungs														
Area(s), dark red	–	–	–	–	2	–	–	N/A	–	N/A	–	–	1	–
Mammary gland														
Mass	N/A	–	N/A	–	N/A	–	N/A	N/A	–	N/A	N/A	N/A	1 [p]	–
Paws														
Scabbing	–	–	–	–	–	–	–	N/A	–	N/A	–	–	1	–
Swollen	–	–	–	–	–	–	–	N/A	–	N/A	–	–	1	–

2.6.7.7I Repeat-Dose Toxicity: A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs (continued)

A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs														
Daily dose (mg/kg)[a]	0 (Control)		0.10		0.14		0.18				0.25			
Number of animals (primary necropsy and early death)	M:3	F:3	M:3	F:3	M:3	F:3	M:2 (ED)	M:0 (MS)	F:1 (ED)	F:0 (MS)	M:2 (ED)	M:4 (MS)	F:3 (ED)	F:3 (MS)
Macroscopic findings (continued)[n]														
Skin														
Open sore(s)	–	–	–	–	–	–	–	N/A	–	N/A	1	–	–	–
Spleen														
Rough surface	–	–	–	–	–	–	–	N/A	–	N/A	–	–	1	–
Small	–	–	–	–	–	–	–	N/A	–	N/A	2	–	1	–
Pale	–	–	–	–	–	–	–	N/A	–	N/A	–	–	1	–
Stomach														
Mucosa, reddened	–	–	–	–	1	–	1	N/A	–	N/A	–	–	3	–
Thymus														
Small	–	–	–	–	–	–	–	N/A	–	N/A	1	1	1	–
Microscopic findings [n]														
Bone marrow, sternum [q]														
Apoptosis	0	0	0	1	1	0	2	N/A	1	N/A	0	N/A	2	N/A
minimal	–	–	–	1	1	–	–	N/A	1	N/A	–	N/A	–	N/A
mild	–	–	–	–	–	–	2	N/A	–	N/A	–	N/A	2	N/A
Hypocellular	0	0	0	0	0	0	2	N/A	0	N/A	0	N/A	2	N/A
Mild	–	–	–	–	–	–	1	N/A	–	N/A	–	N/A	2	N/A
Moderate	–	–	–	–	–	–	1	N/A	–	N/A	–	N/A	–	N/A
Brain														
Degeneration, trigeminal sensory tract	0	0	0	0	0	0	1	N/A	0	N/A	0	N/A	0	N/A
Minimal	–	–	–	–	–	–	1	N/A	–	N/A	–	N/A	–	N/A

2.6.7.7I Repeat-Dose Toxicity: A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs (continued)

A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs														
Daily dose (mg/kg)[a]	0 (Control)		0.10		0.14		0.18					0.25		
Number of animals (primary necropsy and early death)	M:3	F:3	M:3	F:3	M:3	F:3	M:2 (ED)	M:0 (MS)	F:1 (ED)	F:0 (MS)	M:2 (ED)	M:4 (MS)	F:3 (ED)	F:3 (MS)
Microscopic findings (continued)[n]														
Dorsal root ganglion[r]														
Degeneration, neuronal	0	0	1	1	3	1	2	N/A	1	N/A	2	N/A	3	N/A
Minimal	–	–	–	1	2	1	–	N/A	–	N/A	–	N/A	–	N/A
Mild	–	–	1	–	1	–	–	N/A	–	N/A	–	N/A	–	N/A
Moderate	–	–	–	–	–	–	–	N/A	–	N/A	2	N/A	3	N/A
Severe	–	–	–	–	–	–	2	N/A	1	N/A	–	N/A	–	N/A
Degeneration, nerve fiber	0	0	0	0	0	0	1	N/A	1	N/A	2	N/A	0	N/A
Minimal	–	–	–	–	–	–	1	N/A	1	N/A	–	N/A	–	N/A
Mild	–	–	–	–	–	–	–	N/A	–	N/A	1	N/A	–	N/A
Moderate	–	–	–	–	–	–	–	N/A	–	N/A	1	N/A	–	N/A
Intestines														
Apoptosis	0	0	0	0	0	0	2	N/A	1	N/A	0	N/A	2	N/A
Minimal	–	–	–	–	–	–	–	N/A	1	N/A	–	N/A	–	N/A
Mild	–	–	–	–	–	–	1	N/A	–	N/A	–	N/A	–	N/A
Moderate	–	–	–	–	–	–	1	N/A	–	N/A	–	N/A	2	N/A
Hemorrhage	0	0	0	0	0	0	2	N/A	1	N/A	0	N/A	3	N/A
Minimal	–	–	–	–	–	–	1	N/A	–	N/A	–	N/A	1	N/A
Mild	–	–	–	–	–	–	1	N/A	1	N/A	–	N/A	1	N/A
Moderate	–	–	–	–	–	–	–	N/A	–	N/A	–	N/A	1	N/A
Intussusception	0	0	0	0	0	0	1	N/A	0	N/A	2	N/A	1	N/A

2.6.7.7I Repeat-Dose Toxicity: A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs (continued)

A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs														
Daily dose (mg/kg)[a]	0 (Control)		0.10		0.14		0.18					0.25		
Number of animals (primary necropsy and early death)	M:3	F:3	M:3	F:3	M:3	F:3	M:2 (ED)	M:0 (MS)	F:1 (ED)	F:0 (MS)	M:2 (ED)	M:4 (MS)	F:3 (ED)	F:3 (MS)
Microscopic findings (continued)[n]														
Intestines														
Regenerative hyperplasia	0	0	0	0	0	0	2	N/A	1	N/A	0	N/A	1	N/A
Minimal	–	–	–	–	–	–	–	N/A	–	N/A	–	N/A	1	N/A
Mild	–	–	–	–	–	–	2	N/A	1	N/A	–	N/A	1	N/A
Lymph nodes														
Lymphoid depletion	0	0	0	0	0	0	2	N/A	1	N/A	2	N/A	3	N/A
Minimal	–	–	–	–	–	–	–	N/A	–	N/A	1	N/A	–	N/A
Mild	–	–	–	–	–	–	–	N/A	–	N/A	–	N/A	1	N/A
Moderate	–	–	–	–	–	–	2	N/A	1	N/A	–	N/A	2	N/A
Severe	–	–	–	–	–	–	–	N/A	–	N/A	1	N/A	–	N/A
Peyer's patch														
Lymphoid depletion	0	0	0	0	0	0	2	N/A	1	N/A	2	N/A	2	N/A
Mild	–	–	–	–	–	–	–	N/A	1	N/A	2	N/A	–	N/A
Moderate	–	–	–	–	–	–	1	N/A	–	N/A	–	N/A	2	N/A
Severe	–	–	–	–	–	–	1	N/A	–	N/A	–	N/A	–	N/A
Lymphoid necrosis	0	0	0	0	0	0	1	N/A	1	N/A	0	N/A	3	N/A
Minimal	–	–	–	–	–	–	1	N/A	1	N/A	–	N/A	–	N/A
Mild	–	–	–	–	–	–	–	N/A	–	N/A	–	N/A	1	N/A
Moderate	–	–	–	–	–	–	–	N/A	–	N/A	–	N/A	2	N/A

2.6.7.7I Repeat-Dose Toxicity: A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs (continued)

A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs														
Daily dose (mg/kg)[a]	0 (Control)		0.10		0.14		0.18					0.25		
Number of animals (primary necropsy and early death)	M:3	F:3	M:3	F:3	M:3	F:3	M:2 (ED)	M:0 (MS)	F:1 (ED)	F:0 (MS)	M:2 (ED)	M:4 (MS)	F:3 (ED)	F:3 (MS)
Microscopic findings (continued)[n]														
Peripheral autonomic ganglion (end organ)														
Degeneration, neuronal	N/A	N/A	N/A	N/A	N/A	1	2	N/A	1	N/A	2	N/A	3	N/A
Minimal	N/A	N/A	N/A	N/A	N/A	1	1	N/A	1	N/A	1	N/A	2	N/A
Mild	N/A	N/A	N/A	N/A	N/A	–	1	N/A	–	N/A	1	N/A	1	N/A
Sciatic nerve														
Degeneration	0	0	1	0	0	0	2	N/A	0	N/A	2	N/A	1	N/A
Minimal	–	–	1	–	–	–	2	N/A	–	N/A	1	N/A	1	N/A
Mild	–	–	–	–	–	–	–	N/A	–	N/A	1	N/A	–	N/A
Spinal cord														
Degeneration, axonal, dorsal columns	0	0	1	0	2	0	2	N/A	1	N/A	2	N/A	1	N/A
Minimal	–	–	1	–	2	–	1	N/A	1	N/A	–	N/A	1	N/A
Mild	–	–	–	–	–	–	1	N/A	–	N/A	2	N/A	–	N/A
Spleen														
Lymphoid depletion	0	0	0	0	1	0	2	N/A	1	N/A	0	N/A	2	N/A
Minimal	–	–	–	–	1	–	–	N/A	–	N/A	–	N/A	–	N/A
Mild	–	–	–	–	–	–	–	N/A	1	N/A	–	N/A	–	N/A
Moderate	–	–	–	–	–	–	2	N/A	–	N/A	–	N/A	1	N/A
Severe	–	–	–	–	–	–	–	N/A	–	N/A	–	N/A	1	N/A

2.6.7.7I Repeat-Dose Toxicity: A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs (continued)

A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs														
Daily dose (mg/kg)[a]	0 (Control)		0.10		0.14		0.18					0.25		
Number of animals (primary necropsy and early death)	M:3	F:3	M:3	F:3	M:3	F:3	M:2 (ED)	M:0 (MS)	F:1 (ED)	F:0 (MS)	M:2 (ED)	M:4 (MS)	F:3 (ED)	F:3 (MS)
Microscopic findings (continued)[n]														
Spleen														
Lymphoid necrosis	0	0	0	0	0	0	1	N/A	1	N/A	0	N/A	2	N/A
Minimal	–	–	–	–	–	–	1	N/A	1	N/A	–	N/A	1	N/A
Mild	–	–	–	–	–	–	–	N/A	–	N/A	–	N/A	1	N/A
Stomach														
Ulceration	0	0	0	0	0	0	1	N/A	0	N/A	0	N/A	0	N/A
Moderate	–	–	–	–	–	–	1	N/A	–	N/A	–	N/A	–	N/A
Sural nerve														
Degeneration	0	0	0	0	0	0	2	N/A	0	N/A	2	N/A	1	N/A
Minimal	–	–	–	–	–	–	2	N/A	–	N/A	–	N/A	1	N/A
Mild	–	–	–	–	–	–	–	N/A	–	N/A	2	N/A	–	N/A
Sympathetic ganglion														
Degeneration, neuronal	0	0	0	2	3	2	2	N/A	1	N/A	2	N/A	3	N/A
Minimal	–	–	–	2	3	2	–	N/A	–	N/A	–	N/A	–	N/A
Moderate	–	–	–	–	–	–	–	N/A	–	N/A	2	N/A	1	N/A
Severe	–	–	–	–	–	–	2	N/A	1	N/A	–	N/A	2	N/A
Degeneration, nerve fiber	0	0	0	0	0	0	0	N/A	0	N/A	1	N/A	0	N/A
Minimal	–	–	–	–	–	–	–	N/A	–	N/A	1	N/A	–	N/A

2.6.7.7I Repeat-Dose Toxicity: A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs (continued)

A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs														
Daily dose (mg/kg)[a]	0 (Control)		0.10		0.14		0.18				0.25			
Number of animals (primary necropsy and early death)	M:3	F:3	M:3	F:3	M:3	F:3	M:2 (ED)	M:0 (MS)	F:1 (ED)	F:0 (MS)	M:2 (ED)	M:4 (MS)	F:3 (ED)	F:3 (MS)
Microscopic findings (continued)[n]														
Thymus														
Lymphoid depletion	0	0	0	0	0	0	2	N/A	1	N/A	2	N/A	3	N/A
Moderate	–	–	–	–	–	–	–	N/A	–	N/A	–	N/A	1	N/A
Severe	–	–	–	–	–	–	2	N/A	1	N/A	2	N/A	2	N/A
Lymphoid necrosis	0	0	0	0	0	0	1	N/A	0	N/A	0	N/A	2	N/A
Minimal	–	–	–	–	–	–	1	N/A	–	N/A	–	N/A	1	N/A
Mild	–	–	–	–	–	–	–	N/A	–	N/A	–	N/A	1	N/A
Bone marrow cytology														
Number of animals (bone marrow cytology)	M:3	F:3	M:3	F:3	M:3	F:3	M:1		F:1		M:2		F:1	
Cell degeneration	–	–	–	–	–	–	+		+		+		+	
Cellularity														
Low	–	–	–	–	–	–	–		+		–		–	
Very low	–	–	–	–	–	–	–		–		+		–	
Extremely low	–	–	–	–	–	–	+		–		+		+	
Increased M:E	–	–	–	–	+	+	+		+		+		+	
Increased lysis	–	–	–	–	–	–	–		+		+		+	
Neutrophilic left shift	–	–	–	–	–	–	–		+		+		+	
Increased macrophages	–	–	–	–	–	+	–		–		–		–	

2.6.7.7I Repeat-Dose Toxicity: A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs (continued)

A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs										
Daily dose (mg/kg)[a]	0 (Control)		0.10		0.14		0.18		0.25	
Number of animals (recovery)	M:2	F: 2	M:3	F:3	M:0	F:0	M:4	F: 5	M:0	F:0
Recovery necropsy (Day 46)										
Body weights, clinical observations, neurological assessment, hematology, coagulation, and clinical chemistry	All findings were reversed or reversing during the recovery period.									
Organ weights and macroscopic findings	All findings were reversed or reversing during the recovery period.									
Microscopic findings										
Brain										
Degeneration, white matter, trigeminal sensory tract	0	0	0	0	N/A	N/A	2	3	N/A	N/A
Minimal	–	–	–	–	N/A	N/A	1	1	N/A	N/A
Mild	–	–	–	–	N/A	N/A	1	2	N/A	N/A
Dorsal root ganglion [p]										
Degeneration, nerve fiber	0	0	1	0	N/A	N/A	4	4	N/A	N/A
Minimal	–	–	1	–	N/A	N/A	2	1	N/A	N/A
Mild	–	–	–	–	N/A	N/A	2	2	N/A	N/A
Moderate	–	–	–	–	N/A	N/A	–	1	N/A	N/A
Degeneration, neuronal	0	0	0	0	N/A	N/A	0	1	N/A	N/A
Mild	–	–	–	–	N/A	N/A	–	1	N/A	N/A

2.6.7.7I Repeat-Dose Toxicity: A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs (continued)

A Repeated-Dose Intravenous Infusion Toxicity Study of MLN2238 With a 14-Day Recovery Period in Beagle Dogs										
Daily dose (mg/kg)[a]	0 (Control)		0.10		0.14		0.18		0.25	
Number of animals (recovery)	M:2	F: 2	M:3	F:3	M:0	F:0	M:4	F: 5	M:0	F:0
Microscopic findings (continued)										
Peripheral autonomic ganglion (end organ)										
Degeneration, neuronal	N/A	N/A	N/A	N/A	N/A	N/A	1	N/A	N/A	N/A
Minimal	N/A	N/A	N/A	N/A	N/A	N/A	1	N/A	N/A	N/A
Sciatic nerve										
Degeneration	0	0	2	0	N/A	N/A	4	5	N/A	N/A
Minimal	–	–	2	–	N/A	N/A	2	2	N/A	N/A
Mild	–	–	–	–	N/A	N/A	2	3	N/A	N/A
Sural nerve										
Degeneration	0	0	0	0	N/A	N/A	4	3	N/A	N/A
Minimal	–	–	–	–	N/A	N/A	4	1	N/A	N/A
Mild	–	–	–	–	N/A	N/A	–	2	N/A	N/A
Spinal cord										
Degeneration, axonal, dorsal columns	0	0	1	0	N/A	N/A	4	5	N/A	N/A
Minimal	–	–	1	–	N/A	N/A	–	–	N/A	N/A
Mild	–	–	–	–	N/A	N/A	2	1	N/A	N/A
Moderate	–	–	–	–	N/A	N/A	2	4	N/A	N/A
Sympathetic ganglions										
Degeneration, neuronal	0	0	0	0	N/A	N/A	1	1	N/A	N/A
Minimal	–	–	–	–	N/A	N/A	1	1	N/A	N/A
Degeneration, nerve fiber	0	0	0	0	N/A	N/A	0	1	N/A	N/A
Minimal	–	–	–	–	N/A	N/A	–	1	N/A	N/A
Bone barrow cyotology	All findings were reversed or reversing during the recovery period.									

* $p < 0.05$, ** $p < 0.01$ (Dunnett test); + = noteworthy findings; – = no noteworthy findings; A:G = ratio of albumin to globulin; ALB = albumin; ALP = alkaline phosphatase; AUC_{24} = area under the plasma concentration-time curve from the time 0 to 24 hours; AUE_{24} = area under the effect-time curve from the time 0 to 24 hours; BIW = twice weekly; CA = calcium; CHOL = cholesterol; C_{max} = maximum observed plasma concentration; ED = early death; E_{max} = maximum effect; F = female(s); FIB = fibrinogen; GLOB = globulin; GLP = Good Laboratory Practice; HNSTD = highest nonseverely toxic dose; I% = percentage of proteasome inhibition; IV = intravenous(ly); LEUK = leukocyte; M = male(s); M:E = myeloid-to-erythroid ratio; MONO = monocyte; MS = main study; N/A = not applicable; NEUT = neutrophil; PHOS = phosphorus; PLT = platelet; RETIC = reticulocyte; TP = total protein; TRIG = triglycerides.

Note: A separate, non-GLP-compliant analysis was performed that determined that dose-limiting toxicity (DLT) was associated with plasma exposure and blood AUE_{24} Report 4.2.3.2-19 and 4.2.3.2-20). Mortality and severe clinical signs were observed in dogs dosed at 0.18 and 0.25 mg/kg (Groups 3 and 4, respectively). Therefore, dosing was suspended on Day 7 (females only) and Day 3, respectively. Surviving dogs in Group 4 were euthanized on Day 12 (females) and Day 16 (females). Surviving dogs in Group 3 did not receive test article for the remainder of the study. Group 5, dosed at 0.14 mg/kg, was formed for Cycle 2, and the values presented were calculated from samples obtained on Day 10 of that cycle (Day 31 of the study).

a Dose levels were 0.10, 0.18, 0.25, and 0.14 mg/kg for Groups 2, 3, 4, and 5, respectively. Dosing was suspended for Group 4 (0.25 mg/kg) males and females following the second dose (Study Day 3) and for Group 3 (0.18 mg/kg) females following the third dose (Study Day 7). Group 3 males and Group 2 (0.10 mg/kg) males and females received all 4 scheduled doses during Cycle 1. All Group 2 dogs (0.10 mg/kg) remained on study and were dosed again at 0.10 mg/kg during Cycle 2. Group 5 was created for Cycle 2, was dosed at 0.14 mg/kg for all 4 scheduled doses during Cycle 2.

b n = 6 for Cycle 1; n = 5 for Cycle 2.

c Due to lack of significant difference between sexes, data is for sexes combined.

d Data from males only as dosing was suspended for females in Group 3 on Study Day 7.

e Two 0.25 mg/kg females and one 0.18 mg/kg male were found dead on Days 4, 5, and 10 following clinical signs of unrelieved gastrointestinal (GI) symptoms.

f One 0.25 mg/kg female and two 0.25 mg/kg males were euthanized on Days 8, 13, and 14 and one 0.18 mg/kg female and one 0.018 mg/kg male were euthanized on Days 8 and 11 following clinical signs of unrelieved GI symptoms.

g +/- indicates presence or absence of finding in each group.

h This is a common finding in laboratory animals, and was not considered to be test article-related in doses ≤ 0.14.

i There were no corresponding clinical or detailed physical examination findings to confirm a test article effect at these dose levels.

j For controls, group means are shown. For ixazomib-dosed groups, percent differences from controls are shown. Statistical significance is based on actual data, not percent differences.

k Leukocyte, neutrophil, and platelet counts increased for females at 0.25 mg/kg because of a single female.

l Primary necropsy was Day 32 for the 0-, 0.10-, and 0.14 mg/kg groups only. Surviving 0.18 mg/kg animals were not necropsied until the end of the recovery period (Day 46). The primary necropsy for the 0.25 mg/kg group was Day 16 for males and Day 12 for females.

m Both absolute and relative weights differed from controls in the direction indicated. Number indicates percent difference for the absolute organ weights.

n Number indicates number of animals with finding present in tissues examined.

o Number of animals with finding in any intestinal segment (cecum, colon, duodenum, ileum, jejunum, or rectum); most severe grade presented; when findings of multiple severity were present in different parts of the intestine, the most severe grade is presented here.

p Tissue was examined for only 1 of 3 animals.

q Similar findings were observed in the femoral bone marrow.

r Number of animals with finding in any dorsal root ganglion (DRG); when findings of multiple severities were present in different DRGs, the most severe grade is presented here.

s Number of animals with finding in any sympathetic ganglion; most severe grade presented.

2.6.7.7J Repeat-Dose Toxicity: A 5-Cycle Intravenous (5-Minute) Infusion Toxicity Study of MLN9708 in Beagle Dogs

Species/strain:	Dog/beagle	Duration of dosing:	94 days (5 cycles of BIW infusions for 2 consecutive weeks separated by a 10-day nondosing period)	Test article:	Ixazomib citrate
Initial age:	Approximately 7 - 8 months	Duration of postdose:	None	Report	4.2.3.2-21
Date of first dose:	[redacted] (Males) [redacted] (Females)	Day of first dose (Day 0 or Day 1):	Day 0	GLP compliance:	Yes
Method of administration:	Intravenous (IV) infusion (5-minute)				
Vehicle/formulation:	0.9% Sodium Chloride For Injection, USP				
Special features:	Pharmacodynamic analysis (20S proteasome activity, assessed by fluorometry) and blood pharmacokinetic (PK) analysis were done in a separate, non-GLP-compliant study (Report 4.2.3.2-22).				
Study endpoint:	HNSTD = 0.15 mg/kg				

A 5-Cycle Intravenous (5-Minute) Infusion Toxicity Study of MLN9708 in Beagle Dogs

Daily dose (mg/kg)[a]	0 (Control)		0.05		0.10		0.15	
Number of animals	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Plasma toxicokinetics[b]								
C_{max} (ng/mL)								
Day 0	N/A		237		358		538	
Day 94	N/A		273		380		526	
AUC_{24} (ng·hr/mL)								
Day 0	N/A		265		439		790	
Day 94	N/A		688		1380		1470	

2.6.7.7J Repeat-Dose Toxicity: A 5-Cycle Intravenous (5-Minute) Infusion Toxicity Study of MLN9708 in Beagle Dogs (continued)

A 5-Cycle Intravenous (5-Minute) Infusion Toxicity Study of MLN9708 in Beagle Dogs								
Daily dose (mg/kg)[a]	0 (Control)		0.05		0.10		0.15	
Number of animals	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Pharmacodynamics[b]								
E_{max} (I%)								
Day 0	N/A		45.4		55.6		59.3	
Day 94	N/A		43.5		56.2		61.2	
AUE_{24} (hr·I%)								
Day 0	N/A		284		358		476	
Day 94	N/A		223		436		575	
Noteworthy findings								
Body weight (kg)[c, d]	11.2	10.5	+4.5	−1.9	+6.3	+1.0	0.0	−5.7
Hematology and Coagulation[d]								
MON, abs (10^3/μL)								
Week 1	0.57	0.47	+15.8	−12.8	+10.5	+21.3	+82.5	+70.2*
Week 7	0.53	0.57	+43.4	−31.6	+11.3	−22.8	+60.4	+43.9*
Week 13	0.68	0.53	−5.9	−35.8	−2.9	−17.0	+39.7	+60.4
NEUT, abs (10^3/μL)								
Week 1	5.47	5.78	+7.1	−4.5	−6.8	+4.5	+70.9**	+43.8
Week 7	5.66	6.17	+15.0	−23.7	−15.7	−18.5	+38.3	+38.4
Week 13	6.56	6.99	−13.1	−34.9	+3.4	−30.2	+73.2	+33.8
WBC (thous/μL)								
Week 1	9.45	10.18	+18.5	+1.3	−2.3	+0.4	+49.3*	+24.8
Week 7	9.48	9.93	+20.5	−6.1	−9.3	−13.6	+32.8	+30.6
Week 13	10.41	10.89	−6.4	−23.1*	+2.8	−22.7*	+51.3	+24.6*
FIB (mg/dL)								
Week 1	171.3	160.0	+20.8	+26.9	+33.3	+52.1	+49.4	+39.8
Week 7	166.0	179.0	+39.6	−12.8	−3.4	+1.7	+39.2	+51.6
Week 13	193.7	166.0	−0.4	−7.0	+12.2	+12.0	+24.8	+44.8
Clinical chemistry[d]								
Phosphorous (mg/dL)								
Week 1	6.9	7.0	−4.3	−12.9*	−10.1	−17.1**	−21.7*	−14.3*
Week 7	6.0	4.9	−10.0	+10.2	−15.0	+6.1	−25.0**	0.0
Week 13	5.3	4.9	−5.7	0.0	−20.8	−6.1	−28.3	−12.2

2.6.7.7J Repeat-Dose Toxicity: A 5-Cycle Intravenous (5-Minute) Infusion Toxicity Study of MLN9708 in Beagle Dogs (continued)

A 5-Cycle Intravenous (5-Minute) Infusion Toxicity Study of MLN9708 in Beagle Dogs								
Daily dose (mg/kg)[a]	0 (Control)		0.05		0.10		0.15	
Number of animals	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Urinalysis[d]								
Corrected total phosphorous (mg/kg)								
Week 1	12.90	20.32	+62.2	–8.7	–35.7	–0.2	+65.0	+24.1
Week 7	6.35	11.60	+158.4	+69.2	+202.2	+77.6	+255.1*	+147.6
Week 13	7.13	9.94	+50.5	+139.2	–20.3	+127.7	+135.3	+162.5
Total phosphorous (mg)								
Week 1	136.30	188.09	+60.4	–8.1	–33.3	–2.8	+63.8	+23.5
Week 7	72.48	103.16	+148.0	+94.6	+188.6	+101.5	+228.3*	+163.5
Week 13	82.60	103.30	+48.8	+126.1	–19.0	+130.1	+119.8	+154.5
Primary necropsy (Day 95)								
Microscopic findings[e]								
Brain								
Degeneration, white matter tract, medulla	0	0	0	0	0	0	1	0
Minimal	–	–	–	–	–	–	1	–
Dorsal root ganglion[g]								
Degeneration, neuronal	0	0	0	0	3	3	3	3
Minimal	–	–	–	–	–	3	2	2
Mild	–	–	–	–	3	–	1	1
Degeneration, nerve fiber	1	0	0	2	3	3	3	3
Minimal	1	–	–	2[f]	2	3	–	1
Mild	–	–	–	–	1	–	3	2
Intestine[h]								
Infiltration, neutrophil	0	0	0	0	1	1	1	1
Minimal	–	–	–	–	1	1	1	1
Lymph node[i]								
Necrosis, lymphoid	0	0	0	0	1	1	3	3
Minimal	–	–	–	–	1	1	3	3
Perianal (hepatoid) gland								
Single cell necrosis	0	0	0	0	0	0	1	2
Minimal	–	–	–	–	–	–	1	2

2.6.7.7J Repeat-Dose Toxicity: A 5-Cycle Intravenous (5-Minute) Infusion Toxicity Study of MLN9708 in Beagle Dogs (continued)

A 5-Cycle Intravenous (5-Minute) Infusion Toxicity Study of MLN9708 in Beagle Dogs

Daily dose (mg/kg)[a]	0 (Control)		0.05		0.10		0.15	
Number of animals	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Peripheral autonomic ganglion (intestine)[h]								
Degeneration, neuronal	0	0	0	0	3	2	3	3
Minimal	–	–	–	–	3	2	3	3
Peripheral autonomic ganglion (salivary gland)[j]								
Degeneration, neuronal	0	0	0	0	1	1	3	3
Minimal	–	–	–	–	1	1	2	3
Mild	–	–	–	–	–	–	1	–
Peyer's patch								
Infiltration, neutrophil	0	0	0	0	1	1	2	3
Minimal	–	–	–	–	1	1	1	2
Mild	–	–	–	–	–	–	1	1
Sciatic nerve[k]								
Degeneration	0	0	1	1	3	2	3	3
Minimal	–	–	1	1	3	2	1	2
Mild	–	–	–	–	–	–	2	1
Spinal cord								
Degeneration, axonal, dorsal columns	0	0	0	0	3	2	3	3
Minimal	–	–	–	–	2	1	1	1
Mild	–	–	–	–	1	1	2	2
Sural nerve[k]								
Degeneration	0	0	1	0	1	0	2	1
Minimal	–	–	1	–	1	–	1	1
Mild	–	–	–	–	–	–	1	–
Sympathetic ganglion[l]								
Degeneration, neuronal	0	0	0	0	3	3	3	3
Minimal	–	–	–	–	1	3	–	2
Mild	–	–	–	–	2	–	3	1
Degeneration, nerve fiber	0	0	0	0	0	0	2	2
Minimal	–	–	–	–	–	–	2	2

2.6.7.7J Repeat-Dose Toxicity: A 5-Cycle Intravenous (5-Minute) Infusion Toxicity Study of MLN9708 in Beagle Dogs (continued)

A 5-Cycle Intravenous (5-Minute) Infusion Toxicity Study of MLN9708 in Beagle Dogs								
Daily dose (mg/kg)[a]	0 (Control)		0.05		0.10		0.15	
Number of animals	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Vagus nerve								
Degeneration	0	0	0	0	0	0	3	1
Minimal	–	–	–	–	–	–	3	1

*p < 0.05, **p < 0.01 (Dunnett test); + = noteworthy findings; – = no noteworthy findings; abs = absolute; AUC_{24} = area under the plasma concentration-time curve from the time 0 to 24 hours; AUE_{24} = area under the effect-time curve from time 0 to 24 hours; BIW = twice-weekly; C_{max} = maximum observed plasma concentration; E_{max} = maximum effect; F = female(s); FIB = fibrinogen; GLP = Good Laboratory Practice; HNSTD = highest nonseverely toxic dose; I% = percentage of proteasome inhibition; M = male(s); MON = monocyte; N/A = not applicable; NEUT = neutrophil; USP = United States Pharmacopeia; WBC = white blood cell.

a Doses are presented in terms of mg/kg ixazomib.

b There were no pronounced or consistent sex-related differences in exposure to ixazomib; therefore, toxicokinetic (TK) and pharmacodynamic data are presented as sexes combined

c At the end of the dosing period.

d For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not percent differences).

e Number indicates total number of animals with finding present in examined tissue.

f Both animals in this group had focal, minimal change in the ventral spinal root (1 animal) or mixed spinal nerve (1 animal).

g Number of animals with lesion in cervical and/or lumbar dorsal root ganglia (DRG); when severity of findings differed amongst sites, the highest level of severity is presented.

h Number of animals with lesion in any section of intestine (including ileum, jejunum, and/or rectum); when severity of findings differed amongst sites, the highest level of severity is presented.

i Number of animals with lesion in any lymph node (mesenteric, mandibular, and/or popliteal); when severity of findings differed amongst sites, the highest level of severity is presented.

j Number of animals with lesion in either mandibular salivary gland; when severity of findings differed amongst sites, the highest level of severity is presented.

k Number of animals with lesion in left and/or right nerve; when severity of findings differed amongst sites, the highest level of severity is presented.

l Number of animals with lesion in abdominal and/or thoracic ganglia; when severity of findings differed amongst sites, the highest level of severity is presented.

2.6.7.8 In Vitro 遺伝毒性試験

2.6.7.8A Genotoxicity-In Vitro: Salmonella-E Coli/Mammalian Microsome Reverse Mutation Assay

Test for induction of:	Reverse mutation in bacterial cells	Number of independent assays:	2	Test article:	Ixazomib
Strains:	*S. typhimurium* TA1537, TA98, TA100, and TA1535 and *E. coli* WP2*uvrA*	Number of replicate cultures:	3	Report:	4.2.3.3.1-1
Metabolizing system:	Aroclor 1254-induced rat liver S9, 7.5% (v/v)	Number of cells analyzed/culture:	N/A	GLP compliance:	Yes
Vehicles:	For test article: DMSO			Date of treatment:	Initial assay: [redacted]
					Confirmatory assay: [redacted]
	For positive controls: DMSO				
Treatment:	Plate incorporation for 48 hours				
Cytotoxic effects:	Reduction in background growth or spontaneous revertants at ≥ 100 µg/mL (TA100 + S9); at ≥ 250 µg/mL (TA100 and TA1537 –S9, TA1535 and WP2*uvr*A + S9); ≥ 500 µg/mL (TA1537 + S9); and ≥ 1000 µg/mL (TA98, TA1535, and WP2*uvr*A -S9), and ≥ 2500 µg/plate (TA98 + S9)				
Genotoxic effects:	None				

Initial Assay

Metabolic Activation	Test Article	Concentration (µg/plate)	Revertant Colonies per Plate (Mean ± SEM) TA98	TA100	TA1535	TA1537	WP2uvrA
Without activation	DMSO	50 µL	10 ± 0	96 ± 3	14 ± 1	8 ± 1	32 ± 4
	Ixazomib	25	13 ± 2	80 ± 2	11 ± 2	8 ± 3	32 ± 3
		50	15 ± 2	73 ± 4	8 ± 0	8 ± 2	30 ± 3
		100	11 ± 1	60 ± 2	12 ± 3	9 ± 2	17 ± 2
		250	11 ± 3	41 ± 5	7 ± 1	5 ± 1	23 ± 2
		500	7 ± 1	27 ± 5	7 ± 0	2 ± 1	22 ± 2
		1000	6 ± 2	17 ± 2	6 ± 1	1 ± 1	16 ± 2
		2500[a]	2 ± 2	0 ± 0	0 ± 0	0 ± 0	10 ± 3
		5000[b]	0 ± 0	0 ± 0	0 ± 0	0 ± 0	8 ± 3
	ICR	0.5	N/A	N/A	N/A	354 ± 42	N/A
	2-NF	2.5	842 ± 59	N/A	N/A	N/A	N/A
	SA	1.0	N/A	548 ± 43	384 ± 5	N/A	N/A
	NQNO	2.0	N/A	N/A	N/A	N/A	944 ± 21

ICR = ICR-191; 2-NF = 2-nitrofluorene; DMSO = dimethyl sulfoxide; GLP = Good Laboratory Practice; N/A = not applicable; NQNO = 4-nitroquinoline-N-oxide; SA = sodium azide; SEM = standard error of the mean; v/v = volume/volume.

a Reduced background in strains TA1537, TA100 and TA1535, plates not counted.; b Reduced background in strain TA1537, TA98, TA100 and TA1535, plates not counted.

2.6.7.8A Genotoxicity-In Vitro: Salmonella-E Coli/Mammalian Microsome Reverse Mutation Assay (continued)

			Initial Assay				
			Revertant Colonies per Plate (Mean ± SEM)				
Metabolic Activation	Test Article	Concentration (µg/plate)	TA98	TA100	TA1535	TA1537	WP2uvrA
With activation	DMSO	50 µL	14 ± 3	102 ± 3	12 ± 1	8 ± 1	39 ± 1
	Ixazomib	25	15 ± 3	72 ± 2	8 ± 0	7 ± 0	26 ± 4
		50	19 ± 2	74 ± 7	9 ± 2	5 ± 1	26 ± 4
		100	18 ± 2	48 ± 1	6 ± 1	6 ± 2	26 ± 4
		250	15 ± 1	23 ± 4	8 ± 3	5 ± 1	17 ± 1
		500	14 ± 2	18 ± 1	6 ± 1	4 ± 2	16 ± 2
		1000	10 ± 1	18 ± 1	3 ± 2	3 ± 1	14 ± 2
		2500[c]	4 ± 1	21 ± 6	0 ± 0	1 ± 0	7 ± 1
		5000[a]	0 ± 0	0 ± 0	0 ± 0	0 ± 0	N/A
	2AA	2.5	2143 ± 22	942 ± 20	193 ± 12	94 ± 3	N/A
		10.0	N/A	N/A	N/A	N/A	562 ± 33

2AA = 2-aminoanthracene; DMSO = dimethyl sulfoxide; GLP = Good Laboratory Practice; N/A = not applicable; SEM = standard error of the mean

a Reduced background in strain TA1537, TA98, TA100 and TA1535, plates not counted; c Reduced background in strain TA1535, plates not counted

2.6.7.8A Genotoxicity-In Vitro: Salmonella-E Coli/Mammalian Microsome Reverse Mutation Assay (continued)Confirmatory Assay

Metabolic Activation	Test Article	Concentration (µg/plate)	Revertant Colonies per Plate (Mean ± SEM) TA98	TA100	TA1535	TA1537	WP2uvrA
Without activation	DMSO	50 µL	12 ± 2	102 ± 11	15 ± 2	6 ± 3	29 ± 2
	Ixazomib	10	N/A	86 ± 2	N/A	N/A	N/A
		25	N/A	84 ± 4	13 ± 1	5 ± 1	N/A
		50	N/A	74 ± 11	16 ± 5	5 ± 0	21 ± 3
		100	12 ± 3	60 ± 1	17 ± 4	4 ± 1	26 ± 4
		250	14 ± 3	48 ± 11	11 ± 2	3 ± 2	17 ± 1
		500	12 ± 0	28 ± 2	10 ± 2	1 ± 1	16 ± 2
		1000	2 ± 1	N/A	5 ± 1	1 ± 1	14 ± 2
		2500	3 ± 1	N/A	N/A	N/A	7 ± 1
		5000[a]	0 ± 0	N/A	N/A	N/A	N/A
	ICR	0.5	N/A	N/A	N/A	311 ± 14	N/A
	2-NF	2.5	749 ± 49	N/A	N/A	N/A	N/A
	SA	1.0	N/A	572 ± 34	476 ± 29	N/A	N/A
	NQNO	2.0	N/A	N/A	N/A	N/A	757 ± 41
With activation	DMSO	50 µL	17 ± 2	102 ± 5	11 ± 3	7 ± 1	39 ± 4
	Ixazomib	10	N/A	89 ± 8	N/A	N/A	N/A
		25	N/A	70 ± 9	8 ± 2	5 ± 1	N/A
		50	N/A	65 ± 8	8 ± 1	6 ± 1	28 ± 1
		100	12 ± 3	46 ± 1	7 ± 1	8 ± 2	23 ± 3
		250	12 ± 1	27 ± 6	5 ± 1	5 ± 1	18 ± 3
		500	6 ± 1	30 ± 8	4 ± 2	2 ± 1	14 ± 5
		1000	9 ± 2	N/A	3 ± 1	0 ± 0	16 ± 3
		2500	1 ± 0	N/A	N/A	N/A	9 ± 3
		5000[a]	0 ± 0	N/A	N/A	N/A	N/A
	2AA	2.5	2005 ± 106	1615 ± 156	204 ± 12	298 ± 17	N/A
		10.0	N/A	N/A	N/A	N/A	509 ± 66

2AA = 2-aminoanthracene; 2-NF = 2-nitrofluorene; DMSO = dimethyl sulfoxide; GLP = Good Laboratory Practice; N/A = not applicable; NQNO = 4-nitroquinoline-N-oxide; SA = sodium azide; SEM = standard error of the mean; v/v = volume/volume.

a Reduced background in strain TA98, plates not counted.

2.6.7.8B Genotoxicity-In Vitro: In Vitro Chromosome Aberration Test in Cultured Human Peripheral Blood Lymphocytes

Test for induction of:	Chromosome aberrations in human peripheral blood lymphocytes (HPBL)	Number of independent assays:	3-hour with and without activation: 1 each 22-hour: 1	Test article:	Ixazomib citrate
Strains:	HPBLs	Number of replicate cultures:	2	Report:	4.2.3.3.1-2
Metabolizing system:	Aroclor 1254-induced rat liver S9	Number of cells analyzed/culture:	Structural chromosomal aberrations: 100 metaphase or 15 aberrant. Numerical aberrations: 200	GLP compliance:	Yes
Vehicles:	For test article: Sterile, distilled water			Date of treatment:	[redacted] (repeat)
	For positive controls: DMSO				
Treatment:	Short (3-hour) and long (22-hour) incubations				
Cytotoxic effects:	Cytotoxicity observed as follows (with % Mitotic reduction): a) 3-hour treatment without metabolic activation, 0.010 µg/mL (3%), 0.050 µg/mL (9%) and 0.10 µg/mL (57%); b) 3-hour treatment with activation, 35 µg/mL (no cytotoxicity), 40 µg/mL (no cytotoxicity) and 45 µg/mL (56%); and c) 22-hour treatment without metabolic activation, 0.050 µg/mL (9%), 0.065 µg/mL (34%), 0.075 µg/mL (45%) and 0.080 µg/mL (58%).				
Genotoxic effects:	Positive for inducing structural aberrations with and without metabolic activation; no statistically significant increases in numerical aberrations (polyploidy or endoreduplication).				

2.6.7.8B Genotoxicity-In Vitro: In Vitro Chromosome Aberration Test in Cultured Human Peripheral Blood Lymphocytes (continued)

3-Hour Incubation							
Metabolic Activation	Test Article	Concentration (µg/mL)	% Mitotic Reduction	% Cells With Aberrations	% Cells With >1 Aberration	% Endoreduplicated Cells	% Polyploid Cells
Without activation	Sterile water (1%)	N/A	0	1.5	0.0	0.0	0.0
	Mitomycin C	0.8	51	73.2*	46.3*	0.0	0.3
	Ixazomib citrate	0.010	3	0.0	0.0	0.0	0.0
		0.050	9	1.5	0.5	0.0	0.0
		0.10	57	11.5*	1.5	0.0	0.5
With activation	Sterile water (1%)	N/A	0	0.5	0.0	0.0	0.5
	Cyclophosphamide	20	18	23.6*	2.4	0.0	0.8
	Ixazomib citrate	35	–3	5.5*	1.0	0.0	0.5
		40	–15	5.0**	1.0	0.0	0.3
		45	56	18.5*	6.8*	0.0	0.5
22-Hour Incubation							
Metabolic Activation	Test Article	Concentration (µg/mL)	% Mitotic Reduction	% Cells With Aberrations	% Cells With >1 Aberration	% Endoreduplicated Cells	% Polyploid Cells
Without activation	Sterile water (1%)	N/A	0	0.5	0.0	0.0	0.0
	Mitomycin C	0.3	60	65.2*	32.6*	0.0	0.0
	Ixazomib citrate	0.050	9	100.0*	100.0*	–[a]	–[a]
		0.065	34	0.0	0.0	0.0	0.3
		0.075	45	6.0*	1.0	0.0	0.8
		0.080	58	9.5*	3.0**	0.0	0.5

* = $p \leq 0.01$ using Fisher Exact Test; ** = $p \leq 0.05$ using Fisher Exact Test.

DMSO = dimethyl sulfoxide; GLP = Good Laboratory Practice; N/A = not applicable.

a Chromosomes pulverized; unable to score for polyploidy and endoreduplication.

2.6.7.9 In Vivo 遺伝毒性試験

2.6.7.9A Genotoxicity-In Vivo: MLN9708: A Single-Dose Oral (Gavage) Bone Marrow Micronucleus Evaluation in CD 1 Mice

Test for induction of:	Bone marrow micronuclei	Treatment schedule:	Single dose	Test article:	Ixazomib citrate
Species/strain:	Mice/ Crl:CD-1 (ICR)	Sampling time:	24 to 28 hours after dosing (all dose groups) and 44 to 48 hours after dosing (0 and 8 mg/kg dose groups)	Report:	4.2.3.3.2-1
Age:	7 - 8 Weeks	Method of administration:	Oral gavage	GLP compliance:	Yes
Cells evaluated:	Erythrocytes in bone marrow; polychromatic erythrocytes (PCEs) and normochromatic erythrocyte (NCEs)			Date of dosing:	[redacted]
Number of cells analyzed/animal:	2000	Vehicle/formulation:	[redacted] and 3% (w:w) glycine, [redacted]		

Special features: The MTD was 6 mg/kg.

Toxic/cytotoxic effects: One male dosed at 8 mg/kg was found dead on Day 2; the body of this animal was noted to be cool to the touch on Day 1. During the range-finding phase, 1 of 3 animals dosed at 8 mg/kg was also found dead on Day 2.

Genotoxic effects: None

Evidence of exposure: Overt toxicity (ie, death) at 8 mg/kg.

Toxicokinetic (TK) analysis was done in a non-GLP-compliant study in a separate set of mice at the same doses, in the same vehicle, at a higher dose volume (8 mL versus 5 mL) (Report 4.2.3.1-1). All animals dosed at 8 mg/kg were euthanized moribund at 6 hours postdose. C_{max} at 2 and 4 mg/kg was 108 and 219 ng/mL, respectively, and AUC_{24} was 557 and 926 ng·hr/mL, respectively.

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

Test Article	Dose (mg/kg) [a]	Animals Used for Micronucleus Evaluation	Mean % MN PCEs (± SD) 24 to 28 Hours Postdose	Mean % MN PCEs (± SD) 44 to 48 Hours Postdose	Mean PCE:TE Ratio (± SD) 24 to 28 Hours Postdose	Mean PCE:TE Ratio (± SD) 44 to 48 Hours Postdose
Vehicle	0	5+5	0.05 ± 0.05	0.03 ± 0.04	0.61 ± 0.04	0.62 ± 0.06
Ixazomib citrate	2	5	0.07 ± 0.03	N/A	0.63 ± 0.05	N/A
	4	5	0.11 ± 0.13	N/A	0.69 ± 0.07	N/A
	8	5+5	0.15 ± 0.08	0.09 ± 0.10	0.67 ± 0.08	0.54 ± 0.06
CP	60	5	0.89 ± 0.35*	N/A	0.57 ± 0.04	N/A

* $p \leq 0.05$ (Dunnett test). AUC_{24} = area under the plasma concentration-time curve from the time 0 to 24 hours; C_{max} = maximum observed plasma concentration; CP = cyclophosphamide monohydrate; GLP = Good Laboratory Practice; MTD = maximum tolerated dose; MN = micronucleated; N/A = not applicable; SD = standard deviation; TE = total erythrocytes (PCE + NCE); w:w = weight-to-weight ratio.

a All doses are presented in terms of mg/kg ixazomib.

2.6.7.9B Genotoxicity-In Vivo: Comet Assay of MLN9708 for Injection in Mice

Test for induction of:	DNA damage	Treatment schedule:	QD × 2 days	Test article:	Ixazomib citrate
Species/strain:	Mice / Crlj:CD1 (ICR)	Sampling time:	3 hours after the final dose	Report:	4.2.3.3.2-2
Age:	8 weeks	Method of administration:	Oral gavage	GLP compliance:	Yes
Cells evaluated:	Liver and glandular stomach			Date of dosing:	[redacted]
Number of cells analyzed/animal:	100	Vehicle/formulation:	[redacted] and 3% (w:w) glycine, [redacted]		

Special features: None

Toxic/cytotoxic effects: A slight decrease in locomotor activity and a decrease in mean body weights were observed after the first dose at 6 mg/kg. Macroscopic findings were limited to 1 animal dosed at 6 mg/kg, which had 2 dark reddish patches of mucosa.

Genotoxic effects: None

Evidence of exposure: Evidence of overt toxicity at 6 mg/kg.

Comet Assay of MLN9708 FOR INJECTION in Mice

Test Article	Dose (mg/kg)[a]	Animals Used for Evaluation	% Tail DNA (Mean ± SD)	
			Liver	Glandular Stomach
Vehicle	0	5	2.78 ± 0.39	4.82 ± 0.19
Ixazomib citrate	0.5	5	NE	NE
	1	5	NE	NE
	2	5	2.73 ± 0.21	4.60 ± 0.67
	4	5	2.76 ± 0.23	4.65 ± 0.10
	6	5	2.62 ± 0.40	4.81 ± 0.56
EMS[b]	200	5	16.50 ± 2.17*	19.24 ± 1.52*

* $p \leq 0.01$ (Student t-test). DNA = deoxyribonucleic acid; EMS = ethyl methanesulfonate; GLP = Good Laboratory Practice; NE = not evaluated; QD = once daily; SD = standard deviation; w:w = weight-to-weight ratio.

a Doses presented in terms of mg/kg ixazomib.

b Ethyl methanesulfonate, Positive control.

2.6.7.10 がん原性試験

該当なし。

2.6.7.11 生殖発生毒性試験：重要な試験以外の試験

2.6.7.11 Reproductive and Developmental Toxicity: Nonpivotal Studies

Test Article: Ixazomib citrate

Species/ Strain	Method of Administration (Vehicle/Formulation)	Dose[a] (mg/kg)	Dosing Period	Number per Group	Noteworthy Findings	Report
Sprague-Dawley rats	Oral gavage [redacted] and 0.45% (w/v) NaCl, [redacted]	0, 0.1, 0.3, 0.4[b], 0.6, and 0.8[b]	0, 0.1, and 0.3 mg/kg: QD on GD 6 - 17. 0.4[b], 0.6, and 0.8[b] mg/kg: Q3D on GD 6, 9, 12, and 15	2 - 6 main study + 3 TK	QD administration: The NOAEL was determined to be 0.1 mg/kg for dams and > 0.3 mg/kg for fetuses. Q3D administration: At 0.8 mg/kg, 1 dam was found dead; 1 dam was euthanized moribund on gestation day 7, with pale liver and stomach distention noted. The NOAEL was determined to be 0.4 mg/kg for both dams and fetuses. No fetal anomalies were noted in any groups.	4.2.3.5.2-1

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

		Administered Daily (QD)			Administered Every 3 Days (Q3D)		
Daily dose (mg/kg)		0	0.1	0.3	0.4	0.6	0.8
Dams	Toxicokinetics (n = 2)						
	C_{max} (ng/mL)	NE	10	29	21	67	NE
	AUC_{24} (ng·hr/mL)	NE	184	348	291	553	NE
	AUC_{72} (ng·hr/mL)	NE	NE	NE	651	1081	NE
	Number pregnant	6	6	6	3	6	2
	Number died or euthanized moribund	–	–	–	–	–	2
	Number aborted or with total resorption of litter	–	–	–	–	1	NE

2.6.7.11 Reproductive and Developmental Toxicity: Nonpivotal Studies (continued)

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

		Administered Daily (QD)			Administered Every 3 Days (Q3D)		
Daily dose (mg/kg)		0	0.1	0.3	0.4	0.6	0.8
Dams (continued)	Clinical observations						
	Abnormal gait	–	–	–	–	1	–
	Black colored stools	–	–	1	–	–	–
	Decreased feces	–	–	1	–	3	–
	Decreased locomotor activity	–	–	–	–	–	1
	Hypothermia	–	–	–	–	–	1
	Sanguineous discharge from vagina	–	–	–	–	1	–
	Soiled fur	–	–	–	–	2	–
	Body weight	–	–	↓* (GD 16 - 20)	–	↓* (GD 8 - 10, 14 - 20)	NE
	Food consumption	–	–	↓* (GD 6 - 16)	↓** (GD 6)	↓ (GD 6**, 12*)	↓** (GD 6)
	Number of corpora lutea	16.8 ± 2.8	15.5 ± 1.2	15.8 ± 1.2	16.7 ± 0.6	15.8 ± 4.1	NE
	Number of implants	15.5 ± 1.6	13.2 ± 2.9	14.5 ± 1.0	16.0 ± 1.7	14.2 ± 1.3	NE
	Post-implantation loss (%)	2.1 ± 3.3	5.6 ± 6.9	7.8 ± 9.2	2.4 ± 4.1	25.2 ± 40.2	NE
Fetuses	Number viable fetuses	15.2 ± 1.6	12.3 ± 2.4*[c]	13.3 ± 1.2*[c]	15.7 ± 2.3	10.7 ± 6.0	NE
	Fetal body weight (g)						
	Males	3.76 ± 0.18	3.67 ± 0.24	3.49 ± 0.51	3.54 ± 0.07	3.20 ± 0.55**	NE
	Females	3.60 ± 0.23	3.38 ± 0.33	3.24 ± 0.57	3.47 ± 0.13	3.02 ± 0.43*	NE

↓: decrease; * $p \leq 0.05$ (Williams test); ** $p \leq 0.05$ (Shirley-Williams test); – = no test article-related effects; AUC_{24} = area under the concentration-time curve from the time 0 to 24 hours; AUC_{72} = area under the concentration-time curve from the time 0 to 72 hours; Cmax = maximum observed concentration; GD = gestation day; NE = not examined; NOAEL = no observed adverse effect level; QD = once daily; Q3D = once every 3 days; TK = toxicokinetic(s); w/v = weight-to-volume ratio.

Note: Data are presented as mean ± standard deviation (SD).

a Doses are presented in terms of mg/kg ixazomib.

b Two pregnant animals dosed at 0.8 mg/kg were found dead or euthanized moribund after the first dose. The remaining 3 animals in this group had not yet received test article; therefore, dosing at 0.8 mg/kg was not continued and the remaining animals were reassigned to the 0.4 mg/kg group.

c Not judged to be toxicologically significant given the lower numbers of corpora lutea and implants that were not affected by dosing from Gestation Days 6 through 17.

2.6.7.12 生殖発生毒性試験：受胎能及び着床までの初期胚発生に関する試験

該当なし。

2.6.7.13 生殖発生毒性試験：胚・胎児発生に関する試験

2.6.7.13A Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development: Effects of MLN9708 on Embryo-Fetal Development in Rats

Design similar to ICH S5(R2) 4.1.3.:	Yes	Duration of dosing:	10 Days (Q3D dosing on GD 6, 9, 12, and 15)	Test article:	Ixazomib citrate
Species/strain:	Sprague-Dawley rat	Day of mating:	Day 0	Reports:	4.2.3.5.2-2
Initial age:	Approximately 12 weeks	Day of C-section:	Day 20	GLP compliance:	Yes
Date of first dose:	[redacted]	Method of administration:	PO	Vehicle/formulation:	[redacted] and 0.45w/v% NaCl, [redacted]
Special features:	None				

No observed adverse effect level

F0 Females: < 0.2 mg/kg (general toxicity) / ≥ 0.6 mg/kg (reproductive toxicity)

F1 Litters: ≥ 0.6 mg/kg

Effects of MLN9708 on Embryo-Fetal Development in Rats

Daily dose (mg/kg) [a]		0 (Control)	0.2	0.4	0.6
Dams	Toxicokinetics (n = 3/time point)				
	C_{max} (ng/mL)				
	GD 6 (first dose)	N/A	6	22	46
	GD 15	N/A	12	33	49
	AUC_{24} (ng·hr/mL)				
	GD 6 (first dose)	N/A	99	246	428
	GD 15	N/A	201	346	511
	AUC_{72} (ng·hr/mL)				
	GD 6 (first dose)	N/A	275	590	964
	GD 15	N/A	489	738	1103
	Number pregnant	20	20	19	20
	Number died or euthanized moribund	0	0	0	2 [b]
	Number aborted or with total resorption of litter	0	0	0	0

2.6.7.13A Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development: Effects of MLN9708 on Embryo-Fetal Development in Rats (continued)

Effects of MLN9708 on Embryo-Fetal Development in Rats					
Daily dose (mg/kg) [a]		0 (Control)	0.2	0.4	0.6
Dams (continued)	Clinical observations				
	Decreased feces	–	–	–	+
	Soiled fur	–	–	–	+
	Body weight (g) [c, d]	418	–3.6*	–4.5*	–7.9*
	Food consumption (g/animal/day) [d]				
	GD6	25	–12*	–44*	–64*
	GD15	28	–18†	–46†	–54†
	Macroscopic findings in dams				
	Discoloration in stomach	–	–	–	1
	Small thymus	–	–	1	11
	Mean number corpora lutea	17.1	15.8	15.9	16.2
	Mean number implantations	15.3	14.9	15.2	14.8
	Mean preimplantation loss per litter (%)	9.5	5.4	3.9	8.5
Litters	Number litters evaluated	20	20	19	18
	Mean number viable fetuses	14.8	14.2	14.6	13.7
	Mean number placental remnants	0.5	0.8	0.6	1.1
	Mean number dead fetuses	0.1	0	0	0.1
	Mean postimplantation loss per litter (%)	3.3	5.1	3.9	7.4
	Mean fetal body weight (g) (d)	3.6	0.0	0.0	-5.5
	Male	3.7 g	–	1.1	–3.8
	Female	3.5 g	–	–1.7	–4.3
	Sex ratio (M / [M + F][%])	47.7	49.4	53.3	50.3
	Placental and fetal anomalies	There were no test article-related placental or fetal anomalies.			

* $p \leq 0.05$ (Williams Test); † $p \leq 0.05$ (Shirley-Williams Test); – = no noteworthy findings; AUC_{24} = area under the plasma concentration-time curve from the time 0 to 24 hours; AUC_{72} = area under the plasma concentration-time curve from the time 0 to 72 hours; Cmax = maximum observed plasma concentration; C-section = Cesarean section;
F = female(s); F0 = first generation; F1 = second generation; GD = gestation day; GLP = Good Laboratory Practice; ICH = International Conference on Harmonisation; M = male(s); N/A = not applicable; PO = oral(ly); Q3D = once every 3 days.

a Doses presented as mg/kg ixazomib.

b Animals 4F11 and 4F10 were found dead on Days 7 and 8 of gestation, respectively. Black discoloration of the stomach was observed in Animal 4F10. The deaths were considered test article-related because animals dosed with 0.8 mg/kg in a separate range-finding study were also found dead or were euthanized moribund on GD 7.

c GD 20.

d For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

2.6.7.13B Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development: An Oral (Gavage) Dose Range-Finding Study of MLN9708 on Embryo/Fetal Development in Rabbits

Design similar to ICH S5(R2) 4.1.3.:	No	Duration of dosing:	13 Days (GD 7, 10, 13, 16, and 19)	Test article:	Ixazomib citrate
Species/strain:	Rabbit/New Zealand white	Day of mating:	Day 0	Report:	4.2.3.5.2-3
Initial age:	Approximately 5.5 months	Day of C-section:	Day 29	GLP compliance:	Yes
Date of first dose:	[redacted]	Method of administration:	Oral gavage	Vehicle/formulation:	[redacted] and 3% glycine, [redacted]

Special features: Pharmacodynamic analysis (20S proteasome activity in whole blood, assessed by fluorometry) was done in a non-GLP-compliant analysis.
No observed effect level: 0.5 mg/kg for embryo-fetal effects

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

Daily dose (mg/kg) [a]		0 (Control)	0.25	0.5	1.0	1.2
Dams	Toxicokinetics (n = 2 or 3/time point)					
	C_{max} (ng/mL)					
	GD 7 (first dose)	N/A	7.82	13.0	17.5	23.8
	GD 19	N/A	10.9	18.1	33.4	66.6
	AUC_{72} (ng·hr/mL)					
	GD 7 (first dose)	N/A	426	785	1070	1390
	GD 19	N/A	549	956	1340	1600

2.6.7.13B Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development: An Oral (Gavage) Dose Range-Finding Study of MLN9708 on Embryo/Fetal Development in Rabbits (continued)

An Oral (Gavage) Dose Range-Finding Study of MLN9708 on Embryo/Fetal Development in Rabbits						
Daily dose (mg/kg) [a]		0 (Control)	0.25	0.5	1.0	1.2
Dams (continueds)	Pharmacodynamics (n = 3/time point):					
	E_{max} (I%)					
	GD 7 (first dose)	N/A	11.8	12.4	9.7	15.2
	GD 19	N/A	10.8	8.2	14.9	17.6
	AUE_{72} (hr·I%)					
	GD 7 (first dose)	N/A	313.5	245.1	447.5	800.9
	GD 19	N/A	325.0	241.3	495.9	579.4
	Number pregnant	5	6	5	6	6
	Number died or euthanized moribund	0	0	0	0	3
	Number aborted or with total resorption of litter	0	0	0	0	0
	Clinical observations [b]					
	Hair loss	–	–	–	–	16/2
	Decreased defecation	5/2	2/2	29/5	30/6	42/6
	Diarrhea	–	–	1/1	8/5	5/4
	Soft stool	–	–	2/2	7/4	7/4
	Body weight (%) [c,d]	3677	2.0	–4.3	–5.0	–15.0
	Body weight gain (g)[d]					
	GD 7-10	46	–148	–320**	–567**	–663**
	GD 7-22	255	–16	–52	–131**	–176**
	Food consumption (g/animal/day) [d]					
	GD 7-8	162	–40**	–69**	–66**	–73**
	GD 7-22	138	–1.4	-23.9	–33.3	–68.1**
	Mean number of corpora lutea	10.0	10.3	10.6	10.0	9.0
	Mean number of implants	9.8	9.7	9.2	10.0	8.3
	Mean preimplantation loss (%)	1.7	6.1	11.2	0.0	7.0

2.6.7.13B Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development: An Oral (Gavage) Dose Range-Finding Study of MLN9708 on Embryo/Fetal Development in Rabbits (continued)

An Oral (Gavage) Dose Range-Finding Study of MLN9708 on Embryo/Fetal Development in Rabbits						
Daily dose (mg/kg) [a]		0 (Control)	0.25	0.5	1.0	1.2
Litters	Number litters evaluated	5	6	5	6	3
	Mean number viable fetuses	9.4	9.3	9.0	8.5	8.0
	Mean number resorptions	0.4	0.3	0.2	1.5	0.3
	Mean number dead fetuses	0	0	0	0	0
	Mean postimplantation loss per litter (%)	3.5	3.6	2.0	15.4	4.2
	Mean fetal body weight [d] (%)	41.0 g	1.2	1.5	–4.6	1.0
	Fetal anomalies	There were no fetal anomalies				

** $p < 0.01$ (Dunnett test); – = no noteworthy findings; AUC_{72} = area under the plasma concentration-time curve from the time 0 to 72 hours; AUE_{72} = area under the effect-time curve from the time 0 to 72 hours; C_{max} = maximum observed plasma concentration; C-section = Cesarean section; Emax = maximum effect;

GD = Gestation Day; GLP = Good Laboratory Practice; I% = percentage of proteasome inhibition; ICH = International Conference on Harmonisation; N/A = not applicable; Q3D = once every 3 days.

a Doses are presented as mg/kg ixazomib.

b Total occurrence/number of animals.

c GD 22.

d For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

2.6.7.13C Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development: Effects of MLN9708 on Embryo-Fetal Development in Rabbits

Design similar to ICH S5(R2) 4.1.3.:	Yes	Duration of dosing:	13 days (Q3D dosing GD 7, 10, 13, 16, and 19)	Test article:	Ixazomib citrate
Species/strain:	Rabbit/New Zealand white	Day of mating:	Day 0	Report:	4.2.3.5.2-4
Initial age:	16 - 17 weeks	Day of C-section:	GD 28	GLP compliance:	Yes
Date of first dose:	[redacted]	Method of administration:	PO	Vehicle/formulation:	[redacted] and 3% (w:v) glycine, [redacted]
Special features:	None				

No observed adverse effect level
F0 Females: 0.1 mg/kg (general toxicity)/ ≥ 1.0 mg/kg (reproductive function)
F1 Litters: 0.1 mg/kg

Effects of MLN9708 on Embryo-Fetal Development in Rabbits

Daily dose (mg/kg) (a)		0 (Control)	0.1	0.3	1.0
Dams	Toxicokinetics (n = 3/time point)				
	C_{max} (ng/mL)				
	GD 7 (first dose)	N/A	3	11	22
	GD 19	N/A	9	15	29
	AUC_{24} (ng·hr/mL)				
	GD 7 (first dose)	N/A	30	191	443
	GD 19	N/A	189	324	586
	AUC_{72} (ng·hr/mL)				
	GD 7 (first dose)	N/A	162	679	1203
	GD 19	N/A	497	792	1314
	Number pregnant	18 (b)	19 (b)	19	19
	Number died or euthanized moribund	1 (c)	0	0	0
	Number aborted or with total resorption of litter	0	0	1 (d)	0

2.6.7.13C Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development: Effects of MLN9708 on Embryo-Fetal Development in Rabbits (continued)

Effects of MLN9708 on Embryo-Fetal Development in Rabbits					
Daily dose (mg/kg) (a)		0 (Control)	0.1	0.3	1.0
Dams (continued)	Clinical observations (e)				
	Decreased feces	5/2	7/4 (f)	21/13	91/19
	Loose stool	-	-	-	2/2
	Soiled fur	-	-	-	15/3
	Body weight (kg)[g]				
	GD 9	3.38	-1.2	-3.0	-5.9*
	GD 21	3.56	-1.1	-1.4	-3.9*
	GD 28	3.68	-1.1	-0.3	3.3
	Body weight gain (kg)[g]				
	GDs 7 - 9	0.01	-100	-600*	-1800*
	GDs 10 - 12	0.04	-25	-50†	-100†
	GDs 13 - 15	0.05	-20	-20	-100*
	Food consumption (g/animal/day)[g]				
	GD 7	159	-10.7	-59.1†	-89.3†
	GD 9	171	1.2	-1.8	-49.1†
	GD 10	167	-10.8	-18.6*	-74.3*
	GD 13	114	-	-37.7*	-52.6*
	GD 16	142	-11.3	-19.7*	-45.8*
	GD 19	154	-9.7†	-27.9†	-39.6†
	Mean number corpora lutea	9.9	9.4	10.7	8.8
	Mean number implantations	8.5	7.7	9.7	7.4
	Mean preimplantation loss per litter (%)	14.1	18.2	8.9	14.2
Litters	Number litters evaluated	16	18	18	19
	Mean number viable fetuses	8.3	7.3	9.3	6.7
	Mean number placental remnants	0.1	0.3	0.3	0.6†
	Mean number dead fetuses	0.2	0.2	0.1	0.1
	Mean postimplantation loss per litter (%)	2.7	5.8	4.6	8.6

2.6.7.13C Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development: Effects of MLN9708 on Embryo-Fetal Development in Rabbits (continued)

Effects of MLN9708 on Embryo-Fetal Development in Rabbits

Daily dose (mg/kg) (a)		0 (Control)	0.1	0.3	1.0
Litters (continued)	Mean fetal body weight (g)	There were no test article-related effects on fetal body weight.			
	Sex ratio (M / [M + F][%])	There were no test article-related effects on sex ratio.			
	Placental anomalies	There were no test article-related placental abnormalities.			
	Fetal anomalies				
	External abnormalities (%)				
	Short tail	-	-	-	1.8
	Visceral abnormalities	-	-	-	-
	Visceral variations	-	-	-	-
	Skeletal abnormalities (%)				
	Fused caudal vertebrae	-	-	-	3.3
	Unossified caudal vertebrae	-	-	-	0.7
	Skeletal variations (%)				
	Full supernumerary rib	54.6	48.0	75.0	83.7†
	Variation in number of lumbar vertebrae	21.9	13.8	33.7	50.1†
	Number of ossified sacro-caudal vertebrae	There were no test article-related effects on number of ossified sacro-caudal vertebrae.			

* $p \leq 0.05$ (William's test); † $p \leq 0.05$ (Shirley-William's test); - = no noteworthy findings; AUC_{24} = area under the plasma concentration-time curve from the time 0 to 24 hours; AUC_{72} = area under the plasma concentration-time curve from the time 0 to 72 hours; Cmax = maximum observed plasma concentration; C-section = Cesarean section; F = female(s); F0 = first generation; F1 = second generation; GD = gestation day; GLP = Good Laboratory Practice; ICH = International Conference on Harmonisation; M = male(s); N/A = not applicable; PO = oral(ly); Q3D = once every 3 days; w:v = weight-to-volume ratio.

a Doses are presented as mg/kg ixazomib.

b Litter data for 1 dam in each group were excluded from evaluation because identification of the uterus for these dams was not conducted because of human error at necropsy.

c Accidental death on GD 7 due to incubation error.

d Abortion on GD 22.

e Total occurrence/number of animals.

f Finding was not considered test article related in this group because it was sporadic.

g For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

2.6.7.14 生殖発生毒性試験：出生前及び出生後の発生並びに母体の機能に関する試験

該当なし。

2.6.7.15 新生児を用いた試験

該当なし。

2.6.7.16 局所刺激性試験

2.6.7.16 Local Tolerance

Test articles: Ixazomib citrate and ixazomib

Species/Strain	Method of Administration	Test Article	Doses	Sex/Number per Group	Noteworthy Findings	Report
Human	In vitro	Ixazomib	0.361, 3.61, 10.83, 36.1, and 108.3 µg/mL	4/sex	No hemolysis	4.2.3.6-1
Human	In vitro	Ixazomib citrate	1.2 and 2.4 mg/mL	4/sex	No hemolysis	4.2.3.6-2

Note: Local tissue effects were also assessed in the Good Laboratory Practice (GLP)-compliant repeat-dose toxicity studies in Sprague-Dawley rats and beagle dogs. No effects were observed; intestinal toxicity in the oral (PO) studies was related to systemic exposure, and intestinal toxicity was also observed in the intravenous (IV) studies. See Tabulated Summaries 2.6.7.7B (Report 4.2.3.2-4), 2.6.7.7D (Report 4.2.3.2-9), 2.6.7.7F (Report 4.2.3.2-13), and 2.6.7.7H (Report 4.2.3.2-21).

2.6.7.17 その他の毒性試験

2.6.7.17 Other Toxicity Studies

Test Article: Ixazomib citrate

Phototoxicity

Test System	Method of Administration	Duration of Treatment	Doses [a] (μg/mL)	Replicates	Report
BALB/3T3 clone A31 cells	In vitro	1 hour incubation followed by 50 minutes at room temperature, with or without irradiation	0.024, 0.098, 0.391, 1.563, 6.25, 25, 100, or 400	6	4.2.3.7.7-1

Noteworthy findings: Ixazomib citrate did not elicit any phototoxic response under the conditions of this study. The IC_{50} for ixazomib citrate was 30.579 μg/mL in the non-irradiated state and 19.806 μg/mL in the irradiated state, and the PIF value was 1.54. [b]

IC_{50} = concentration producing 50% inhibition; PIF = photo-irritation factor.

a Doses are presented as μg/mL ixazomb.

b PIF = IC_{50} for non-irradiation / IC_{50} for irradiation.

2.6.7.17 Other Toxicity Studies (continued)

	Test Article: Ixazomib citrate
In Silico Mutagenicity Analysis for Drug Substance Synthesis Scheme	Report
Appropriate synthesis control strategies are discussed within Module 3.	–