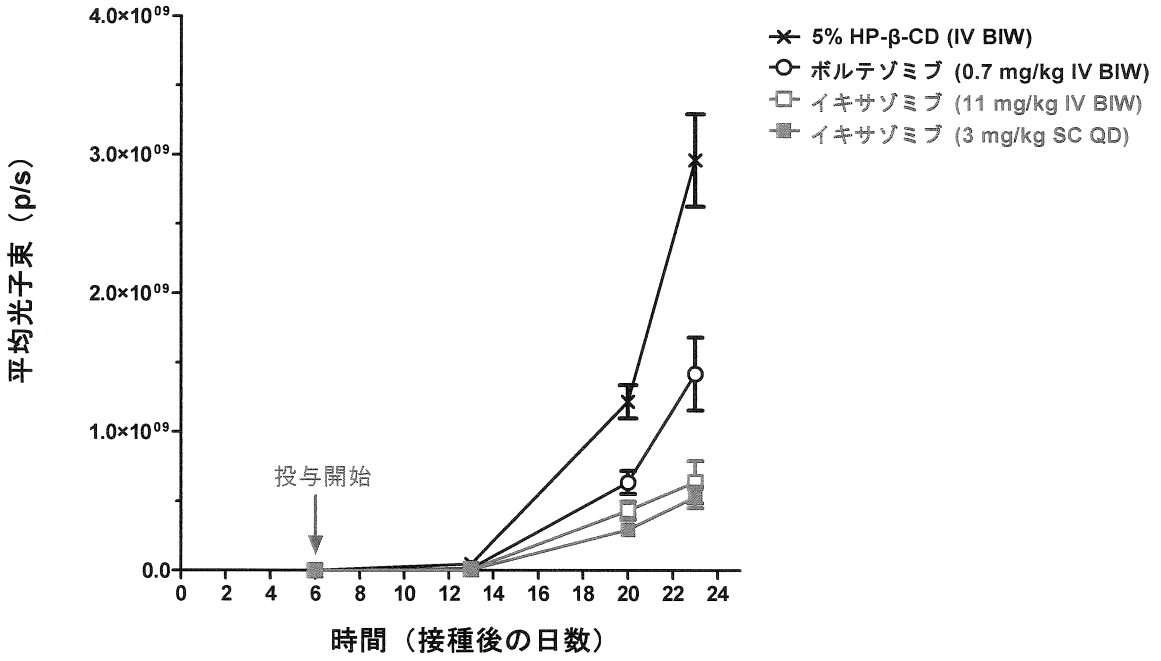


図 2.6.2.2-21　DP54-Luc iMyc^{Ca}/Bcl-XL 形質細胞腫瘍を播種した雌 NOD-SCID マウスモデルにおける腫瘍量

出典：添付資料番号 4.2.1.1-13
BIW = 週 2 回；HP-β-CD = ヒドロキシプロピル-β-シクロデキストリン；IV = 静脈内；MLN2238 = イキサゾミブ；NOD-SCID = 非肥満糖尿病-重症複合免疫不全；p/s = 光子/秒；QD = 1 日 1 回；SC = 皮下
投与は試験開始 6 日目に開始した。データ点は、各投与群の平均光量子束密度±SEM を示す。接種後 20 日目に光量子束密度値を投与群間で比較し、群間差が統計的に有意であるかどうか評価した。対応のない両側 t 検定を用いて、平均光量子束密度の群間差の統計解析を実施した。p 値が ＜0.05 のときに統計的に有意であるとみなした。

イキサゾミブ 11 mg/kg を週 2 回静脈内投与した群（SSSA = 0.85 ± 0.16 mm^2）は、溶媒投与群（SSSA = 1.16 ± 0.04 mm^2）と比べて、頭蓋縫合の離開が 27%有意に縮小した（$p < 0.05$）（図 2.6.2.2-22）。それとは対照的に、ボルテゾミブ 0.7 mg/kg を週 2 回静脈内投与した群は、溶媒投与群に比べて、SSSA（1.17 ± 0.08 mm^2）に有意な影響を及ぼさなかった（$p > 0.05$）。なお、本試験においては、イキサゾミブの 1 日 1 回皮下投与群の SSSA は測定しなかった。イキサゾミブ又はボルテゾミブ投与各群において平均最大体重減少が 5%を越えることはなかった。

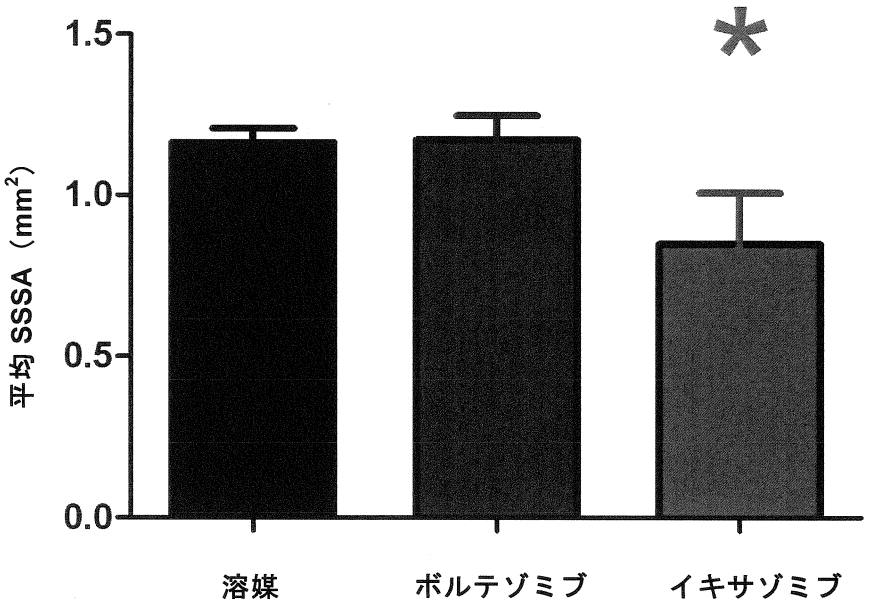


図 2.6.2.2-22　DP54-Luc iMyc^{Cα}/Bcl-XL PCM 形質細胞腫瘍を播種した雌 NOD-SCID マウスモデルにおけるイキサゾミブ投与後の個々の矢状縫合離開面積

出典：添付資料番号 4.2.1.1-13
NOD SCID = 非肥満糖尿病-重症複合免疫不全；SSSA = 矢状縫合離開面積
SSSA（mm²）は、ホルマリンで固定したマウス（接種後 23 日目に安楽死）の CT 画像を用いて測定した；各群 n = 7。一元配置分散分析（ANOVA）を用いて、対数変換後のデータに対して統計解析を実施した。* : $p < 0.05$（統計的に有意とみなした）。

（3）遺伝子改変マウスモデル由来の形質細胞腫瘍 DP54-Luc の脛骨内移植マウスモデルにおける抗腫瘍活性

雌ヌードマウスの脛骨の骨髄腔に DP54-Luc iMyc^{Cα}/Bcl-XL PCM 細胞を接種した。腫瘍量は、[redacted]定量解析が可能な生物発光イメージングで、週 1 回、全光量子束密度を測定してモニタリングした。全光量子束密度がベースライン値に達した時点で、マウスを各投与群にランダムに割り付けた（各群 n = 8）。マウスには、3 週間連続して、HP-β-CD 5%、イキサゾミブ 13 mg/kg、又はボルテゾミブ 0.8 mg/kg を週 2 回静脈内投与した（概要表 2.6.3.2；添付資料番号 4.2.1.1-14）。投与は試験開始 10 日目に開始した。抗腫瘍活性は、29 日目に測定した平均光量子束密度値（腫瘍量）の T/C 比を算出することによって判定した。

イキサゾミブ 13 mg/kg 週 2 回静脈内投与群は、溶媒投与群に比べて有意な抗腫瘍活性を示した（T/C = 0.05、$p < 0.05$）（図 2.6.2.2-23）。ボルテゾミブ 0.8 mg/kg 週 2 回静脈内投与群も同様の抗腫瘍活性を示した（T/C = 0.02、$p < 0.05$）。いずれの投与群でも平均最大体重減少は 5%を超えなかった。

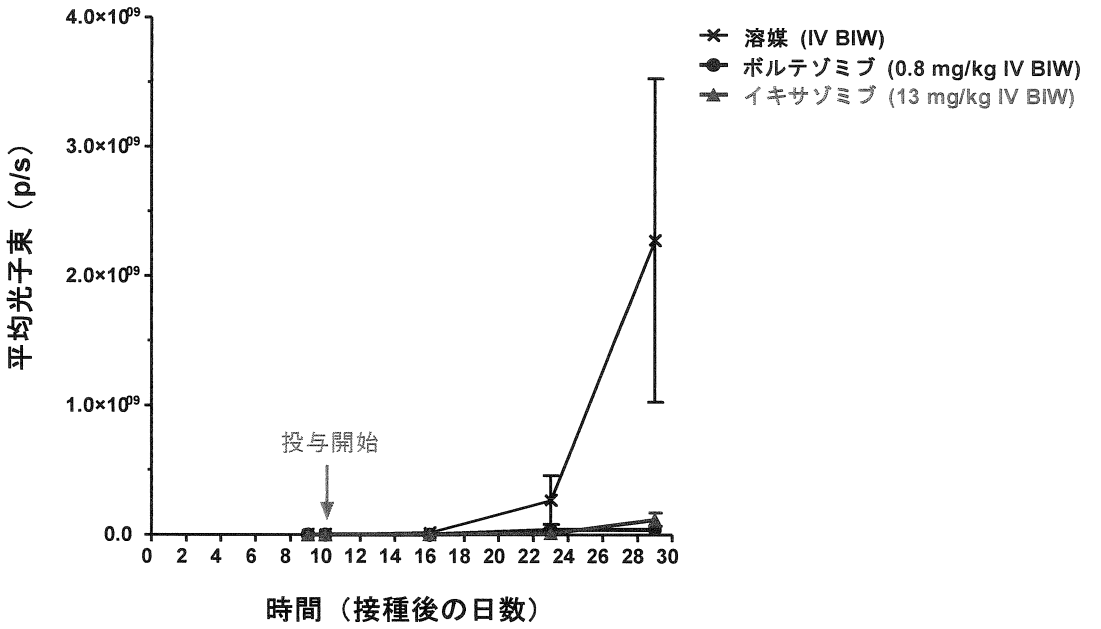


図 2.6.2.2-23　DP54-Luc iMyc$^{C\alpha}$/Bcl-XL 形質細胞を脛骨内移植した雌ヌードマウスモデルにおける平均光量子束密度

出典：添付資料番号 4.2.1.1-14
BIW = 週 2 回；IV = 静脈内；p/s = 光子/秒
投与は試験開始 10 日目に開始した。データ点は、各投与群の平均光量子束密度±SEM を示す。薬物投与群で、$p < 0.05$、対応のない両側 t 検定（ウェルチ検定による補正）。

2.6.2.3 副次的薬理試験

イキサゾミブの副次的な薬理効果については、これまで確認されていないため、副次的薬理試験は実施しなかった。103 種類のキナーゼ及び 18 種類の受容体で構成されるパネルに対するイキサゾミブの選択性について評価した in vitro 試験のデータから、イキサゾミブが off-target 効果をもたらす可能性は低いことが示された（Section 2.6.2.2.1.1）。

2.6.2.4 安全性薬理試験

2.6.2.4.1 in vitro 安全性薬理試験

2.6.2.4.1.1 hERG アッセイ

：添付資料番号 4.2.1.3-1（参考）

hERG によってコードされる遅延整流性カリウム電流（I_{Kr}）の即時活性成分とのイキサゾミブの in vitro での結合作用を HEK-293 細胞を用いて評価した（概要表 2.6.3.4；添付資料番号 4.2.1.3-1）。リガンド（[^{3}H] dofetilide 3.5 nmol/L － 受容体部位の 50%を占めるのに要する濃度 [KD]）及びイキサゾミブ（DMSO 1.0%を用いて最終濃度が 0.00508～100 µmol/L となるように調製）を 96 ウェルプレートに添加して、競合アッセイを実施した。膜ホモジネート（蛋白質 15 µg/well）を添加し、室温でプレートを培養して、反応させた。プレートを乾燥させた後、液体シンチレーションカウンター（LSC）を用いて結果を評価した。いずれの実験も 2 枚のウェルプレートに対して各 2 回実施した。イキサゾミブの作用については、総結合対照（膜及びリガンド）と NSB 対照（膜、リガンド、及び terfenadine 10 µmol/L）と比較して

阻害率を評価した。陽性対照である terfenadine も、イキサゾミブに相当する各濃度で評価した。各濃度における hERG チャネルとの[^{3}H] dofetilide 結合の阻害率を表 2.6.2.4-1 に示す。

表 2.6.2.4-1　イキサゾミブによる[^{3}H] dofetilide 結合の平均阻害率

イキサゾミブ濃度 (μmol/L)	[^{3}H] dofetilide 結合の平均[a]阻害率 (%)
100	65.68
33.3	36.79
11.1	19.55
3.70	11.34
1.23	15.22
0.412	5.09
0.137	8.85
0.0457	5.10
0.0152	4.31
0.00508	11.92

出典：添付資料番号 4.2.1.3-1
a　各数値は 4 ウェルの平均（2 枚のウェルプレートに対してそれぞれ 2 ウェル）

イキサゾミブ（DMSO 1.0%を用いて最終濃度が 0.00508～100 μmol/L となるように調製）は、HEK-293 細胞において、クローン化した心臓のカリウム（K^+）hERG チャネルを阻害したが、その作用は弱かった（K_i = 24.9 μmol/L 及び IC_{50} = 59.6 μmol/L）。

in vitro での hERG 活性と関連するイキサゾミブ濃度（K_i = 24.9 μmol/L）は、第 3 相臨床試験で 4 mg を週 1 回で 3 週間の 28 日サイクルで投与した場合のヒトの平均最高血漿中濃度（C_{max}）約 0.13 μmol/L を 190 倍上回った（ヒト血漿蛋白結合を考慮するとこの倍率はさらに顕著に高くなると思われる）。したがって、イキサゾミブは、臨床的に意義のある用量で hERG チャネルを阻害する可能性は低い。

追加の選択性スクリーニングにおいて、イキサゾミブは 18 種類の受容体（神経伝達物質、イオンチャネル並びに脳及び腸の受容体）で構成されたパネルに対して影響を及ぼさず（IC_{50} は各受容体に対して > 10 μmol/L）、イキサゾミブが off-target 効果をもたらす可能性は低いことが示された（Section 2.6.2.2.1.1）。

2.6.2.4.2 in vivo 安全性薬理試験

ビーグル犬を用いて GLP 準拠で実施したテレメトリー試験において、心血管系にイキサゾミブが及ぼす影響について評価した（概要表 2.6.3.4；添付資料番号 4.2.1.3-2）。ラット及びイヌを用いて GLP 準拠で実施した反復投与毒性試験の一環として、心電図、神経系及び呼吸系の評価も実施した（Section 2.6.6.3）。

2.6.2.4.2.1 ビーグル犬を用いた強制経口投与による心血管テレメトリー試験

：添付資料番号 4.2.1.3-2（評価）

強制経口投与後にイキサゾミブが心拍数、血圧及び ECG パラメータに及ぼす潜在的な影響を特定するため、ラジオテレメトリーの送信器を埋め込んだ無麻酔無拘束ビーグル犬を用いて、GLP に準拠した試験を実施した（概要表 2.6.3.4；添付資料番号 4.2.1.3-2）。実験歴のない雄ビーグル犬 4 匹に、溶媒（0.5% 重量－体積比[w:v]メチルセルロース[MC] 溶液）又はイ

キサゾミブクエン酸エステルの 0.021、0.14、0.21 mg/kg（イキサゾミブとしての投与量）を漸増用量法（各投与量を 7 日間隔）で投与した。血圧、心拍数、ECG 及び体温測定は、投与前 0.5 時間及び投与後 0.5、1、2、4、8、24 時間に実施した。

さらに、雄ビーグル犬を用いた別の試験を実施し、同一の投与量を投与した場合のトキシコキネティクス（TK）を評価した（Section 2.6.6.2.5；概要表 2.6.7.3；添付資料番号 4.2.1.3-3）。C_{max} と AUC_{24} はどちらも用量依存的に上昇した（表 2.6.2.4-2）。0.14 mg/kg 投与後、血漿濃度に二峰性のピークが認められたことから、最高血漿中濃度到達時間（t_{max}）はやや遅延した。なお、この原因を特定することはできなかった（本試験の詳細ついては Section 2.6.6.2.5 で要約する）。

表 2.6.2.4-2　イキサゾミブクエン酸エステルを単回経口投与したビーグル犬におけるイキサゾミブの平均血漿トキシコキネティクスの要約

投与量 (mg/kg)[a]	t_{max} (hr)	C_{max} (ng/mL)	AUC_{24} (ng·hr/mL)
0.021	0.5	10	98
0.14	2.3[b]	75	1047
0.21	0.5	403	2140

出典：添付資料番号 4.2.1.3-3

AUC_{24} = 0 時間から 24 時間の血漿中濃度-時間曲線下面積；C_{max} = 最高血漿中濃度；t_{max} = 最高血漿中濃度到達時間

注記：n = 2

a　イキサゾミブの投与量

b　本投与群の 1 匹の t_{max} が 0.5 時間であるのに対し、他の動物は 4 時間であったが、C_{max} はどちらも同程度であった。

血圧、心拍数又は ECG パラメータ（PR 間隔、QRS 時間、QT 間隔及び補正 QT [QTc] 間隔）に対する本剤投与に関連した影響は、投与量を問わず、いかなるタイムポイントでも観察されなかった。血圧及び PR、QT、QTc 間隔について、統計的に有意な差が散見されたが、これらは溶媒を投与した動物の個体差の範囲内、又は当該動物の投与前の数値の正常変動内であった。したがって、これらの変化は、被験物質に関連しているとはみなさなかった。

結論として、イキサゾミブの単回投与は、最高投与量 0.21 mg/kg まで無麻酔無拘束イヌの心血管系に影響を及ぼさなかった。

2.6.2.4.2.2 反復投与毒性試験における安全性薬理評価

：添付資料番号 4.2.3.2-2（評価）、-4（評価）、-5（評価）、-7（評価）、-9（評価）、-11（評価）、-13（評価）、-15（評価）、-17（評価）、-21（評価）

GLP 準拠で実施した反復投与毒性試験において、イキサゾミブはラット又はイヌの呼吸系に明らかな影響を及ぼさず、生命を脅かす急性の神経系への影響ももたらさなかった。イヌでは、0.10 mg/kg 以上の経口投与（週 2 回での 2 週間の投与期間とそれに続く 10 日間の休薬期間で構成される 21 日サイクル）により、交感神経、後根、末梢自律神経及び末梢器官神経節の変性、並びに脊髄の後柱における上行路及び末梢神経の二次的な軸索／神経線維変性の顕微鏡的な神経学的所見が観察された（Section 2.6.6.3.9）。ときに、病理組織学的所見は、臨床徴候である運動失調、開脚姿勢及び反射低下と相関していた。イヌを用いた 9 ヵ月間毒性試験（10 サイクル）で、臨床レジメン（28 日サイクル）を模して投与したところ、0.20 mg/kg（AUC_{24} 1870 ng·hr/mL）の投与量で、同様の神経細胞の病理組織学的所見が認められた

（Section 2.6.6.3.11）。さらに、神経行動学的な機能評価を実施したが、神経機能の変化は観察されなかった。神経学的所見の大半は、後根神経節（DRG）及び脊髄の所見を除いて、休薬による回復又は回復傾向が認められた。末梢神経節において進行中の神経変性がなかったこと、並びに神経線維及び軸索において二次的な変性変化しか認められなかったことは、持続的な毒性がないことを示している。

イヌを用いてGLP準拠で実施した1、5又は10サイクルの経口投与試験、及び2又は5サイクルの静脈内投与試験では、ECGパラメータへの影響は認められなかった（Section 2.6.6.3.9、2.6.6.3.11、2.6.6.3.13及び2.6.6.3.14）。0.1 mg/kgでの1サイクルの経口投与試験（11日目のC_{max} = 159 ng/mL）では雄イヌにおいてQTc間隔延長を示唆する徴候が認められたが、当該試験の雌イヌ及び2サイクルの静脈内投与試験のすべてのイヌではQTc間隔の延長は観察されなかった。なお、これらのイヌのC_{max}曝露量は、1サイクルの経口投与試験においてQTc間隔延長を示唆する徴候が認められた雄イヌのC_{max}曝露量と同程度又はそれ以上であった（Section 2.6.6.3.9及び2.6.6.3.13）。

全体として、心血管系、呼吸系、又は神経系にイキサゾミブが及ぼす影響は、プロテアソーム阻害薬であるボルテゾミブでも観察されている神経作用に限られていた[13]。

2.6.2.5 薬力学的薬物相互作用試験

本非臨床試験では、イキサゾミブの薬力学的薬物相互作用は評価していない。

2.6.2.6 考察及び結論

イキサゾミブは、20Sプロテアソームのβ5サブユニットに対する強力で、可逆的かつ選択的な阻害薬である。培養細胞にイキサゾミブを投与すると、4 × Ub-Luc レポーターの安定化、NF-κB–ルシフェラーゼレポーターの抑制、ユビキチン化された蛋白質の蓄積、UPRの誘導及びアポトーシスからも明らかなように、プロテアソームが阻害され、その結果として蛋白質ホメオスタシスに影響を及ぼすことが示された。イキサゾミブは、各種の培養又は初代ヒトMM細胞の生存率を低下させ、内皮細胞又は破骨細胞及び骨芽細胞の前駆細胞等の骨髄微小環境を反映する細胞種に影響を及ぼすことが実証された。イキサゾミブとレナリドミドを併用し、in vitroでの生存率を測定した結果、評価した4種類のMM細胞株のうち2種類では相乗作用、残りの2種類では相加作用が認められた。

in vivoでは、ヒトMM.1S多発性MM細胞株移植マウスモデルにおいて、イキサゾミブを経口投与及び静脈内投与した結果、いずれの場合も薬力学的作用及び抗腫瘍活性が認められた。3種類のPCMに関連するマウスモデル、すなわち、デノボPCMである$iMyc^{C\alpha}$/Bcl-XL GEMモデル、DP54-Luc $iMyc^{C\alpha}$/Bcl-XLマウス細胞株の静脈内接種によって作製されたPCM播種マウスモデル、及びDP54-Luc $iMyc^{C\alpha}$/Bcl-XLマウス細胞株の脛骨内接種によって作製された脛骨内接種マウスモデルでも、有意な抗腫瘍活性が認められた。デノボPCMである$iMyc^{C\alpha}$/Bcl-XL GEMモデルにイキサゾミブ及びボルテゾミブを投与すると、全生存期間の延長、脾腫の縮小及びIgG2aレベルの低下が認められた。$iMyc^{C\alpha}$/Bcl-XL PCMの静脈内接種によって作製されたDP54-Luc播種マウスモデル及び脛骨内接種マウスモデルでは、イキサゾミブとボルテゾミブはどちらも腫瘍量を低下させた。しかし、DP54-Luc播種マウスモデルに

おいて溶骨性病変を有意に改善させたのはイキサゾミブのみであった（これは頭蓋縫合の離開が縮小することによって証明された）。

in vitro と in vivo の両方で実施された非臨床安全性薬理試験及び評価により、心血管系、呼吸系及び神経系に及ぼす影響は、主にイヌでみられた顕微鏡的な傷害のみであり、この病変は散発的な神経学的臨床徴候を伴うことが示された。動物でのイキサゾミブに起因するニューロンの顕微鏡的な傷害は、プロテアソーム阻害薬であるボルテゾミブでも報告されており、ボルテゾミブ投与患者では末梢神経障害との関連が認められている。しかし、イキサゾミブでは、別のプロテアソーム阻害薬であるカルフィルゾミブを用いた場合に報告されている、心電図（ECG）検査で認められる心血管系への影響は認められなかった[5]。イキサゾミブは末梢神経系に影響を及ぼす可能性があるものの、イキサゾミブに関する安全性薬理試験の全でのデータから、患者に心血管系、呼吸系、又は中枢神経系に対して影響を及ぼす可能性は低いことが示された。

2.6.2.7 図表

本文中に記載。

2.6.2.8 参考文献一覧

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目次

2.6.3 薬理試験概要表 2
2.6.3.1 薬理試験：一覧表 2
2.6.3.2 効力を裏付ける試験 4
2.6.3.3 副次的薬理試験 21
2.6.3.4 安全性薬理試験 22
2.6.3.5 薬力学的薬物相互作用試験 23

2.6.3 薬理試験概要表

2.6.3.1 薬理試験：一覧表

2.6.3.1 Pharmacology Overview

Type of Study	Test Article	Test System	Method of Administration	Testing Facility	添付資料番号
Primary Pharmacodynamics[a]					
Enzymology, cell biology, inhibitory activity, and selectivity in vitro	Ixazomib and bortezomib	HEK-293, MDA-MB-231-FP4, MDA-MB-231, and Calu-6 cells	In vitro	Millennium	4.2.1.1-1
Potency against the β5 and β2 sites of the proteasome in cultured tumor cells	Ixazomib and bortezomib	HCT-116 cells	In vitro	Millennium	4.2.1.1-2
Selectivity and inhibitory activity in vitro	Ixazomib	Panel of 18 receptors (neurotransmitter, ion channel, and receptors of the brain and gut)	In vitro	[redacted]	4.2.1.1-3
Inhibitory activity by a ixazomib enantiomer	ML00701207	Purified human RBC 20S proteasome evaluated in a peptidase activity enzyme assay	In vitro	Millennium	4.2.1.1-4
In vitro and in vivo selective antitumor activity of ixazomib against multiple myeloma cells.	Ixazomib	MM.1S, MM.1R, RPMI-8226, OPM1, OPM2, H929 and INA-6 human myeloma cell lines, purified patient MM cells, PBMC form healthy adult donors, aBMSC (CD138-) and HUVEC	In vitro	Dana-Farber Cancer Institute	4.2.1.1-5
Effects on in vitro viability of human myeloma cell lines	Ixazomib	ANBL-6, MM.1S, RPMI-8226, and NCI-H929 human myeloma cell lines	In vitro	Millennium	4.2.1.1-6
Preclinical activity of ixazomib in myeloma bone disease.	Ixazomib	Osteoclast differentiated form peripheral blood mononuclear cells, osteoblast differentiated from human mesenchymal stem cells	In vitro	Centro de Investigacion del Cancer, IBMCC	4.2.1.1-7
Effects of ixazomib in combination with lenalidomide on in vitro viability of human myeloma cell lines	Ixazomib and lenalidomide	MM.1S, ANBL-6, RPMI-8226, and NCI-H929 human myeloma cell lines	In vitro	Millennium	4.2.1.1-8
Pharmacokinetics/ pharmacodynamics	Ixazomib	Female CB-17 SCID mice bearing MM.1S multiple myeloma xenografts	IV, PO	Millennium	4.2.1.1-9
Antitumor activity	Ixazomib	Female CB-17 SCID mice bearing MM.1S multiple myeloma xenografts	PO	Millennium	4.2.1.1-10

2.6.3.1 Pharmacology Overview (continued)

Type of Study	Test Article	Test System	Method of Administration	Testing Facility	添付資料番号
	Ixazomib	Female CB-17 SCID mice bearing MM.1S multiple myeloma xenografts	IV	Millennium	4.2.1.1-11
	Ixazomib and bortezomib	Male and female iMyc$^{C\alpha}$/Bcl-X_L hybrid (B6 × FVB/N) F_1 mice (GEMM of de novo PCM)	IV	[redacted]	4.2.1.1-12
	Ixazomib and bortezomib	Female NOD-SCID mice bearing disseminated DP54-Luc iMyc$^{C\alpha}$/Bcl-X_L PCM	IV, SC[a]	Millennium	4.2.1.1-13
Antitumor activity (Continued)	Ixazomib and bortezomib	Female nude mice bearing intratibial DP54-Luc iMyc$^{C\alpha}$/Bcl-X_L PCM	IV	Millennium	4.2.1.1-14
Secondary Pharmacodynamics					
No studies of secondary pharmacodynamics have been conducted.					
Safety Pharmacology					
In vitro CV effects	Ixazomib	hERG potassium channel, HEK-293 cells	In vitro	Millennium	4.2.1.3-1
In vivo CV effects	Ixazomib citrate	Male beagle dogs	PO	[redacted]	4.2.1.3-2
Pharmacodynamic Drug Interactions					
No studies of pharmacodynamic drug interactions have been conducted.					

[redacted]USA); CV = cardiovascular; GEMM = genetically engineered mouse model; IV = intravenous(ly); [redacted]Japan);

Millennium = Millennium Pharmaceuticals, Inc (Cambridge, MA, USA); NOD-SCID = non-obese diabetic, severe combined immunodeficient; PCM = plasma cell malignancy; PO = oral(ly); RBC = red blood cell; SC = subcutaneous(ly); SCID = severe combined immunodeficient; [redacted]USA).

a Bortezomib was not administered SC.

2.6.3.2 効力を裏付ける試験

2.6.3.2 Primary Pharmacodynamics

添付資料番号: 4.2.1.1-1

Enzymology and Cell Biology of Ixazomib			
Assay	Parameter	Test Article	Noteworthy Findings[a]
Biochemical assay, K_i (nmol/L)	β5	Ixazomib	0.93 (0.64 - 1.4, n = 3)
		Bortezomib	0.55 (0.34 - 0.89, n = 3)
	β5i	Ixazomib	0.4
		Bortezomib	0.2
Biochemical assay, IC_{50} (nmol/L)	β5	Ixazomib	3.4 (2.8 - 4.1, n = 3)
		Bortezomib	2.4 (2.0 - 2.9, n = 45)
	β2	Ixazomib	3500
		Bortezomib	1200
	β1	Ixazomib	31
		Bortezomib	24 (15 - 40, n = 12)
Biochemical assay, dissociation $t_{1/2}$ (min)	β5 $t_{1/2}$	Ixazomib	18 (6.8 - 30, n = 3)[b]
		Bortezomib	110 (71 - 150, n = 3)[b]
Biochemical assay, k_{on} ($sec^{-1}M^{-1}$)	β5	Ixazomib	700,000 (450,000 - 940,000, n = 3)[b]
		Bortezomib	195,000 (140,000 - 250,000, n = 3)[b]
Biochemical assay, k_{off} (sec^{-1})	β5	Ixazomib	0.00066 (0.00019 - 0.0011, n = 3)[b]
		Bortezomib	0.00011 (0.000067 - 0.00015, n = 3)[b]
Protease selectivity, IC_{50} (μmol/L)	Chymotrypsin	Ixazomib	> 100 (n = 2)
	Trypsin		> 100
	Elastase		19 (7.8 - 47, n = 3)
	Thrombin		> 100
	Plasmin		> 100
	tPA		> 100
	CFbetaXIIa		> 100
	Cathepsin B		> 100 (n = 2)
	Cathepsin L		> 100
	Panel of 103 kinases[c]		> 10

2.6.3.2 Primary Pharmacodynamics (continued)

添付資料番号: 4.2.1.1-1

Enzymology and Cell Biology of Ixazomib			
Assay	Parameter	Test Article	Noteworthy Findings[a]
Cell-based assay MDA-MB-231 4 × Ub-Luc	Activation of 4 × Ub-Luc reporter, EC_{50} (nmol/L)	Ixazomib	525 (330 - 840, n = 4)
		Bortezomib	310 (230 - 400, n = 29)
	Fold stimulation of 4 × Ub-Luc, E_{max} (nmol/L)[d]	Ixazomib	265 (160 - 370, n = 4)[b]
		Bortezomib	370 (330 - 410, n = 29)[b]
Cell-based assay HEK 293 NFκB-Luc	Inhibition of NFκB-Luc reporter, IC_{50} (nmol/L)	Ixazomib	55 (33 - 91, n = 7)
		Bortezomib	33 (27 - 40, n = 23)
	Max inhibition of NFκB-Luc reporter (%)[d]	Ixazomib	99.3 (99.0 - 99.6, n = 7)[b]
		Bortezomib	99.6 (99.3 - 100, n = 23)[b]
Cell-based assay MDA-MB-231-FP4 WST	Cell viability, LD_{50} (nmol/L)	Ixazomib	75
		Bortezomib	11 (6.9 - 15, n = 6)[b]
Cell-based assay Calu-6 Proteasome-Glo™	Inhibition of β5 proteasome activity, IC_{50} (nmol/L)	Ixazomib	9.7
		Bortezomib	3.0
Cell-based assay Calu-6 Proteasome-Glo™ t = 4 hours, no washout	β5 proteasome activity (%)[d]	Ixazomib	7.1 (3.6 - 10.6, n = 5)[b]
		Bortezomib	3.5 (2.0 - 4.9, n = 5)[b]
Cell-based assay Calu-6 Proteasome-Glo™ t = 4 hours, washout	β5 proteasome activity (%)[d]	Ixazomib	69 (66 - 71, n = 5)[b]
		Bortezomib	20 (18 - 23, n = 5)[b]

CFbetaXIIa = F12 coagulation factor XII (Hageman factor); EC_{50} = concentration producing a half-maximal response; E_{max} = maximum effect; IC_{50} = concentration producing 50% inhibition; K_i = inhibition dissociation constant; k_{off} = dissociation constant; kon = association constant; LD_{50} = dose causing lethality for 50% of the population (in vivo); Max = maximum; t = time; $t_{1/2}$ = half-life; tPA = tissue plasminogen activator; WST = water-soluble tetrazolium.

a Unless otherwise noted, all results are reported as the geometric mean 〔95% confidence interval (CI), number of experiments〕. Data without CIs are single determinations.

b Results are reported as the mean (95% CI, number of experiments). Data without CIs are single determinations.

c Testing was performed by Invitrogen Corporation.

d After exposure to 1 μmol/L ixazomib for 30 minutes.

2.6.3.2 Primary Pharmacodynamics (continued)

添付資料番号: 4.2.1.1-2

In Vitro Potency and Selectivity of Ixazomib in HCT-116 Cells				
Assay	Parameter	Test Article	n	Noteworthy Findings
[redacted]	β5 proteasome site inhibition	Ixazomib	6	0.0075 ± 0.00037 μmol/L[a]
		Bortezomib	3	0.0036 ± 0.00021 μmol/L [a]
	β2 proteasome site inhibition	Ixazomib	4	9.1 ± 18 μmol/L [a,b]
		Bortezomib	6	0.41 ± 0.11 μmol/L [a]
	β5 proteasome site inhibition	Ixazomib	3	10 μmol/L: 98% ± 0.19%[c] 100 μmol/L: 99% ± 0.27%[c]
		Bortezomib	3	10 μmol/L: 100% ± 0.16% [c] 100 μmol/L: 100% ± 0.085%[c]
	β2 proteasome site inhibition	Ixazomib	3	10 μmol/L: 44% ± 9.4% [c] 100 μmol/L: 73% ± 3.6%[c]
		Bortezomib	3	10 μmol/L: 84% ± 1.6% [c] 100 μmol/L: 96% ± 2.3%[c]

a Geometric mean IC_{50} (concentration producing 50% inhibition) ± SEM (standard error) (μmol/L). Plates with IC_{50} estimates that were either below or above the lowest or highest compound concentration in the dilution range on the plate (< 0.0051 μmol/L or > 10 μmol/L, respectively) were excluded from the calculation of the mean.

b The large SEM associated with the β2 IC_{50} estimate reflects the low potency of inhibition of the β2 site by ixazomib.

c Geometric mean percent maximal inhibition ± SEM (%).

2.6.3.2 Primary Pharmacodynamics (continued)

添付資料番号: 4.2.1.1-3

In Vitro Selectivity of Ixazomib

Assay	Concentration	Noteworthy Finding
In vitro selectivity assay for inhibition of a panel of 18 receptors (neurotransmitter, ion channel, and receptors of the brain and gut)	1×10^{-5} mol/L	All IC_{50}s for ixazomib were > 10 µmol/L.

IC_{50} = concentration producing 50% inhibition.

2.6.3.2 Primary Pharmacodynamics (continued)

添付資料番号: 4.2.1.1-4

In Vitro Inhibition of ML00701207

Assay	Parameter	Test Article	Concentration	Noteworthy Finding
Peptidase activity enzyme assay	The β5 proteasome site inhibition	ML00701207	0.005 to 100 µmol/L	IC_{50} for ML00701207was 0.8245 µmol/L with a 95% CI of 0.293.

CI = confidence interval; IC_{50} = concentration producing 50% inhibition.

Additional information: These results indicate that ML00701207 is a weaker inhibitor of the proteasome β5 site than ixazomib 〔IC_{50} = 3.4 nmol/L （添付資料番号: 4.2.1.1-1）〕 by a factor of more than 200-fold.

2.6.3.2 Primary Pharmacodynamics (continued)

添付資料番号: 4.2.1.1-5

Enzymology, Cell Biology and Mechanistic Effects of Ixazomib			
Assay	Parameter	Test Article	Noteworthy Findings
Cell-based assay MM.1S specific fluorogenic peptide t = 3 hours	Inhibition of β5 subunit activity, IC_{50} (nmol/L)	Ixazomib (1.25 – 100 nmol/L)	5
	Inhibition of β2 subunit activity, IC_{50} (nmol/L)	Ixazomib (10 – 10,000 nmol/L)	> 10,000
Cell-based assay MM.1S, INA-6, RPMI-8226, MM.1R. H929, OPM1 and OPM2 Cell viability by MTT assay t = 48 hours	Colorimetric measurement, percent inhibition	Ixazomib (6.25, 12.5, 25, 50 nmol/L)	Concetration dependent, significant reduction of viability ($p < 0.05$; n = 3) MM.1S, MM.1R, NCI929; approximately 50% decrease versus control at 12.5 nmol/L RPMI-8226, INA-6; > 90% decrease versus control at 25 nmol/L OPM1, OPM2; 40 – 50% decrease versus control at 25 nmol/L
Cell-based assay purified patient MM cells (CD138+ selection) from 6 patients Cell viability by CellTiter-Glo™ t = 24 or 48 hours	Cell viability,percent inhibition	Ixazomib (12.5, 25, 50 nmol/L)	Concentration-dependent, significant reduction of viability ($p < 0.001$) 25 nmol/L, 24 hours; approxinately 40% reduction versus control (6/6 patients) 50 nmol/L, 24 hours; > 70% versus control (4/6 patients)
Cell-based assay PBMC form healthy adult donors Cell viability by CellTiter-Glo™ t = 48 hours	Cell viability,percent inhibition	Ixazomib (12.5, 25, 50 nmol/L)	25 nmol/L, 48 hours; < 10% versus control (5/6 donors) approximately 20% versus control (1/6 donors) 50 nmol/L, 48 hours; < 30% versus control (5/6 donors) approximately 40% versus control (1/6 donors)
Cell-based assay MM.1S coculture with BMSC (CD138-) Cell growth assay by [^{3}H]thymidine uptake t = 48 hours	Inhibition of [^{3}H]thymidine uptake	Ixazomib (6.25, 12.5, 25 nmol/L)	12.5 nmol/L; approximately 1/2.5 versus control 25 nmol/L; approximately 1/15 versus control
Mechanistic effects NCI-H929, MM.1S immunoblotting for proteins in apoptotic signaling pathway t = 24 hours	Immunoblot density	Ixazomib (12, 15 nmol/L)	Increase in proteolytic cleavage of caspase-3, caspase-8, caspase-9, and PARP (apoptotic pathway) Increase in protein levels of p53- and p53-regulated genes, including p21 and the pro-apoptotic BH3-only proteins NOXA and PUMA (p53 pathway) Increases in levels of the chaperone protein BiP, increase in the phosphorylation of eIF2alpha, and increases in levels of the transcription factor CHOP (signs of UPR)

2.6.3.2 Primary Pharmacodynamics (continued)

添付資料番号: 4.2.1.1-5

Enzymology, Cell Biology and Mechanistic Effects of Ixazomib			
Assay	Parameter	Test Article	Noteworthy Findings
In vitro angiogenesis assay HUVEC Light microscopy observation t = 8 hours for treatment t = 4 hours for capillary-like tube formation	Quantification of the number of branch points per field of view	Ixazomib (10 nmol/L)	approximately 35% reduction of the number of branch points

2.6.3.2 Primary Pharmacodynamics (continued)

添付資料番号: 4.2.1.1-6

In Vitro Viability Effects of Ixazomib in Human Myeloma Cell Lines

Assay	Cell Lines Evaluated	Noteworthy Finding
[redacted]USA]) to measure cellular ATP after 72 hours of incubation with ixazomib	ANBL-6, MM.1S, RPMI-8226, and NCI-H929[a]	LD_{50} geometric mean ± SEM of 0.0243 µmol/L ± 0.0072, 0.0258 µmol/L ± 0.0360, 0.0059 µmol/L ± 0.0017, and 0.0149 µmol/L ± 0.0007, respectively.

ATP = adenosine 5′-triphosphate; LD_{50} = concentration causing lethality for 50% of cells.

a n = 3, 5, 11, and 5 independent determinations for each cell line, respectively. In each determination, ixazomib was tested at 10 concentrations, in duplicate, using 3-fold serial dilutions in dimethyl sulfoxide (DMSO).

2.6.3.2 Primary Pharmacodynamics (continued)

添付資料番号: 4.2.1.1-7

Enzymology, Cell Biology and Mechanistic Effects of Ixazomib			
Assay	Parameter	Test Article	Noteworthy Findings
Cell-based assay $TRAP^+$ osteoclast formation from PBMC Light microscopy observation t = 21 days	Number of TRAP + cells with 3 or more nuclei	Ixazomib (1, 2.5, 5, 10, 25 nmol/L) bortezomib (1, 2.5, 5, 10, 25 nmol/L)	significant inhibition of osteoclast formation without reducing the total number of cells; ixazomib 2.5 – 10 nmol/L
Cell-based assay PBMC in osteoclastogenic media Bone resorption Light microscopy observation t = 17 days	Area of bone resorption	Ixazomib (2.5, 10, 25 nmol/L) bortezomib (1, 2.5, 5 nmol/L)	Ixazomib; significant reduction of calcium resorption at concentrations of 2.5 nmol/L and above Bortezomib; similar effects on osteoclastogenesis in these assays at lower concentrations.
Cell-based assay Primary MSC Osteoblast differentiation ALP activity (a surrogate marker of early-stage osteoblasts) t = 11 days	Increase in ALP activity	Ixazomib (2.5, 5, 10 nmol/L) bortezomib (1, 2.5, 5 nmol/L)	Dose-dependent increased in the presence of ixazomib or bortezomib, with significantly increased ALP observed at concentrations of ixazomib of 2.5 nmol/L and above
Cell-based assay Primary MSC Matrix mineralization Increase in staining with Alizarin Red (a marker for bone mineralization) t = 21 days	Calorimetric measurement of Alizarin Red	Ixazomib (2,5, 5, 10 nmol/L) bortezomib (1, 2.5, 5 nmol/L)	Increase in the presence of ixazomib or bortezomib,

2.6.3.2 Primary Pharmacodynamics (continued)

添付資料番号: 4.2.1.1-8

In Vitro Viability Effects of Ixazomib and Lenalidomide in Human Myeloma Cell Lines			
Assay	Cell Lines Evaluated	Test Article[a]	Noteworthy Findings
[redacted] USA]) to measure cellular ATP after 120 hours of incubation of cells with ixazomib and lenalidomide, alone or in combination.	MM.1S	Ixazomib	EC_{50}[b] = 23.9 nmol/L (20.9, 27.3)
		Lenalidomide	EC_{50}[b] = > 24,900 nmol/L (N/A[c])
		Ixazomib + lenalidomide	CI = N/A[d] NLB[e] = 0 (N/A) Synergy interpretation: additive
	ANBL-6	Ixazomib	EC_{50}[b] = 17.7 nmol/L (15.9, 19.7)
		Lenalidomide	EC_{50}[b] = 578 nmol/L (382, 876)
		Ixazomib + lenalidomide	CI[e] = 0.45 (p < 0.001) NLB[e] = 21.3 (p < 0.001) Synergy interpretation: synergistic
	NCI-H929	Ixazomib	EC_{50}[b] = 18.3 nmol/L (17.1, 19.5)
		Lenalidomide	EC_{50}[b] = 776 nmol/L (621, 969)
		Ixazomib + lenalidomide	CI[e] = 0.48 (p < 0.001) NLB[e] = 23.6 (p < 0.001) Synergy interpretation: synergistic
	RPMI-8226	Ixazomib	EC_{50}[b] = 7.85 nmol/L (5.43, 10.6)
		Lenalidomide	EC_{50}[b] = 447 nmol/L (195, 1170)
		Ixazomib + lenalidomide	CI[e] = N/A[d] NLB[e] = 18.9 (p < 0.001) Synergy interpretation: additive

ATP = adenosine 5′-triphosphate; CI = combination index; EC_{50} = concentration producing a half-maximal response; N/A = not applicable; NLB = nonlinear blending

a Each test article was tested in a series of concentrations ranging from inactive to maximally effective (up to 25 µmol/L).

b Numbers in parentheses represent the 95% confidence intervals for the EC_{50} values.

c Value is N/A because confidence intervals were very wide due to the inactivity of the compound.

d Value is N/A because CI did not exist because one or both of the compounds did not achieve a 50% reduction of viability.

e Numbers in parentheses represent the p-value as determined by Z-test; if result is 0, p-value is N/A.

Additional information: When $p < 0.05$, combination index is defined as follows: synergy (CI 0-0.7); additivity (CI 0.7-1.3); subadditivity (CI 1.3 - 2); antagonism (CI > 2). If a conclusion cannot be made using CI, the nonlinear blending value (NLB) is used. When $p < 0.05$, NLB is defined as follows: synergy (NLB > 20); additivity (NLB between - 20 and 20); antagonism (NLB < -20). When $p > 0.05$ for CI or NLB, the result is scored as inconclusive

2.6.3.2 Primary Pharmacodynamics (continued)

添付資料番号: 4.2.1.1-9

PK/Pharmacodynamics of Ixazomib After a Single PO or IV Administration in MM.1S Multiple Myeloma Xenograft-Bearing Female CB-17 SCID Mice

		PK Parameters				20S Proteasome Inhibition		
Regimen	Tissue	t_{max} (hr)	C_{max} (ng/mL)[a]	AUC_{24} (ng·hr/mL)[a]	T:P AUC Ratio	t_{max} (hr)	E_{max} (I%)[a]	AUE_{24} (hr*I%)[a]
IV,	Plasma	0.25	1290 ± 182	1100 ± 48.2	N/A	N/A	N/A	N/A
2 mg/kg	Tumor	1.0	902 ± 40.7	15,800 ± 798	14.4	4.0	71.7 ± 1.09	1530 ± 32.8
PO,	Plasma	0.25	1540 ± 379	1680 ± 134	N/A	N/A	N/A	N/A
6 mg/kg	Tumor	4.0	807 ± 48.3	16,100 ± 834	9.6	8.0	60.6 ± 1.63	1210 ± 88.6

AUC_{24} = area under the 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; C_{max} = maximum observed concentration; E_{max} = maximum effect; I% = percentage of inhibition; IV = intravenous(ly); N/A = not applicable;

PK = pharmacokinetic(s); PO = oral(ly); SCID = severe combined immunodeficient; T:P = tumor to plasma AUC; tmax = time to reach C_{max} (for PK) or time to reach E_{max} (for proteasome inhibition and ATF-3 expression).

a Data presented as mean ± standard error; n = 3.

Additional Noteworthy Findings:

Additional pharmacodynamic markers evaluated included ATF-3, GADD34, and cleaved caspase-3. ATF-3 elevation, detected by IHC, was observed in the MM.1S xenograft tumors at 4, 8, and 24 hours after IV and PO administration of ixazomib. Intravenous administration of ixazomib resulted in a maximum increase in ATF-3 levels of 4.92-fold at 8 hours postdose when compared to ATF-3 levels in xenografts from the IV vehicle group. Oral dosing resulted in a maximum increase of 9.26-fold at 8 hours postdose when compared to ATF-3 levels in xenografts from the PO vehicle group. GADD34 levels measured by Western blot were elevated at 4, 8, and 24 hours after IV and PO administration of ixazomib. This timing was similar to that seen with ATF-3 increases; both GADD34 and ATF-3 are markers of the unfolded protein response that occurs after proteasome inhibition. Cleaved caspase-3 levels, as measured by Western blot, were low in the vehicle groups and elevated at 8 and 24 hours after IV and PO administration of ixazomib, indicating apoptosis after an acute dose of ixazomib.

2.6.3.2 Primary Pharmacodynamics (continued)

添付資料番号: 4.2.1.1-10

Antitumor Activity of Ixazomib Dosed PO BIW in MM.1S Multiple Myeloma Xenograft-Bearing CB-17 SCID Female Mice

Treatment	Dose (mg/kg)	Method of Administration/ Frequency	Sex/Number Per Group	Species/Strain	Endpoints	Noteworthy Findings
Vehicle (5% HP-β-CD)	0	PO/BIW (× 5 doses)	Female/8	*Mus musculus*/ CB-17 SCID	TGI[a]	N/A
					ΔAUC	N/A
					BW change[b]	-3.3% (Day 4)
Ixazomib	1	PO/BIW (× 5 doses)	Female/5	*Mus musculus*/ CB-17 SCID	TGI	26.3%
					ΔAUC	$p < 0.05$[c]
					BW change[b]	-2.2% (Day 4)
Ixazomib	2	PO/BIW (× 5 doses)	Female/5	*Mus musculus*/ CB-17 SCID	TGI[a]	9.1%
					ΔAUC	$p > 0.05$[c]
					BW change[b]	-2.6% (Day 4)
Ixazomib	4	PO/BIW (× 5 doses)	Female/5	*Mus musculus*/ CB-17 SCID	TGI[a]	30.8%
					ΔAUC	$p < 0.05$[c]
					BW change[b]	-3.6% (Day 11)
Ixazomib	6	PO/BIW (× 5 doses)	Female/5	*Mus musculus*/ CB-17 SCID	TGI[a]	99.3%
					ΔAUC	$p < 0.001$[c]
					BW change[b]	-5.6% (Day 11)
Ixazomib	8	PO/BIW (× 5 doses)	Female/5	*Mus musculus*/ CB-17 SCID	TGI[a]	99.7%
					ΔAUC	$p < 0.001$[c]
					BW change[b]	-11.2% (Day 11)
Ixazomib	10	PO/BIW (× 5 doses)	Female/5	*Mus musculus*/ CB-17 SCID	TGI[a]	100.0%
					ΔAUC	$p < 0.001$[c]
					BW change[b]	-6.9% (Day 4)

ΔAUC = change in the area under the tumor volume-time curve; BIW = twice weekly; BW = body weight; HP-β-CD = hydroxypropyl-beta-cyclodextrin; N/A = not applicable; PO = oral(ly); SCID = severe combined immunodeficient; TGI = tumor growth inhibition.

a TGI was calculated on Day 19 of the study.

b Results are presented as maximum mean percent body weight change (corresponding study day of measurement); negative values represent a loss in body weight.

c The changes in ΔAUC were assessed using a linear mixed effects regression model; $p < 0.05$ was considered statistically significant.

2.6.3.2 Primary Pharmacodynamics (continued)

添付資料番号: 4.2.1.1-11

Antitumor Activity of Ixazomib Dosed IV BIW and QW in MM.1S Multiple Myeloma Xenograft-Bearing CB-17 SCID Female Mice

Treatment	Dose (mg/kg)	Method of Administration/Frequency	Sex/Number Per Group	Species/Strain	Endpoints	Noteworthy Findings
Vehicle (5% HP-β-CD)	0	IV/QW (× 3 doses)	Female/8	*Mus musculus*/ CB-17 SCID	TGI[a]	N/A
					ΔAUC[b]	N/A
					BW change[c]	-3.1% (Day 6)
Ixazomib	0.5	IV/BIW (× 6 doses)	Female/5[d]	*Mus musculus*/ CB-17 SCID	TGI	34.2%
					ΔAUC	$p < 0.05$
					BW change	-2.2% (Day 6)
Ixazomib	1.0	IV/BIW (× 6 doses)	Female/5	*Mus musculus*/ CB-17 SCID	TGI	46.8%
					ΔAUC	$p < 0.001$
					BW change	-1.7% (Day 6)
Ixazomib	2.5	IV/BIW (× 6 doses)	Female/5	*Mus musculus*/ CB-17 SCID	TGI	94.2%
					ΔAUC	$p < 0.001$
					BW change	-10.0% (Day 13)
Ixazomib	7.0	IV/BIW (× 6 doses)	Female/5	*Mus musculus*/ CB-17 SCID	TGI	99.3%
					ΔAUC	$p < 0.001$
					BW change	-6.8% (Day 13)
Ixazomib	0.82	IV/QW (× 3 doses)	Female/5	*Mus musculus*/ CB-17 SCID	TGI	24.7%
					ΔAUC	$p < 0.001$
					BW change	-2.4% (Day 2)
Ixazomib	1.64	IV/QW (× 3 doses)	Female/5	*Mus musculus*/ CB-17 SCID	TGI	93.2%
					ΔAUC	$p < 0.001$
					BW change	-2.1% (Day 13)
Ixazomib	4.1	IV/QW (× 3 doses)	Female/5	*Mus musculus*/ CB-17 SCID	TGI	97.1%
					ΔAUC	$p < 0.001$
					BW change	-7.3% (Day 16)

2.6.3.2 Primary Pharmacodynamics (continued)

添付資料番号: 4.2.1.1-11

Antitumor Activity of Ixazomib Dosed IV BIW and QW in MM.1S Multiple Myeloma Xenograft-Bearing CB-17 SCID Female Mice

Treatment	Dose (mg/kg)	Method of Administration/Frequency	Sex/Number Per Group	Species/Strain	Endpoints	Noteworthy Findings
Ixazomib	11.47	IV/QW (× 3 doses)	Female/5	*Mus musculus*/ CB-17 SCID	TGI	99.5%
					ΔAUC	$p < 0.001$
					BW change	-11.3% (Day 16)

ΔAUC = change in the area under the tumor volume-time curve; BIW = twice weekly; BW = body weight; HP-β-CD = hydroxypropyl-beta-cyclodextrin; IV = intravenous(ly); N/A = not applicable; QW = once weekly; SCID = severe combined immunodeficient; TGI = tumor growth inhibition.

a TGI was calculated on Day 19 of the study.

b The changes in ΔAUC were assessed using a linear mixed effects regression model; $p < 0.05$ was considered statistically significant.

c Results are presented as maximum mean percent body weight change (corresponding study day of measurement); negative values represent a loss in body weight.

d All days and all animals were included for the ΔAUC analysis.

2.6.3.2 Primary Pharmacodynamics (continued)

In Vivo Antitumor Activity of Ixazomib in a GEMM of De Novo PCM

Group/ Treatment	Dose (mg/kg)	Method of Administration/ Frequency	Sex/ Number per Group	Species/ Strain	Endpoints	Noteworthy Findings
Age-matched nontransgenic	N/A	N/A	Male/5 Female/5	Mus musculus/ hybrid (B6 × FVB/N) F_1	Median overall survival[a]	N/A[b]
					Hazard ratio[c]	N/A
					Mean spleen weight + SEM[c,d]	N/A
					Mean IgG2a levels[e]	1.19×10^5 ng/mL
					BW change[f]	N/A
Untreated control	N/A	N/A	Male/5 Female/5	*Mus musculus*/ iMyc$^{C\alpha}$/Bcl-X_L hybrid (B6 × FVB/N) F_1	Median overall survival[a]	112 days
					Hazard ratio[c]	N/A
					Mean spleen weight + SEM[c,d]	0.87 ± 0.07 g
					Mean IgG2a levels[e]	3.79×10^6 ng/mL
					BW change[f]	-7.6% (Day 65)
Bortezomib	1.2	IV/BIW (× 12 doses)	Male/14 Female/16	*Mus musculus*/ iMyc$^{C\alpha}$/Bcl-X_L hybrid (B6 × FVB/N) F_1	Median overall survival[a]	139 days
					Hazard ratio[c]	0.163 (0.085 to 0.312); $p < 0.0001$
					Mean spleen weight + SEM[c,d]	0.59 ± 0.05 g; $p < 0.05$
					Mean IgG2a levels[e]	3.52×10^5 ng/mL; $p < 0.05$
					BW change[f]	41% (Day 203)
Ixazomib	18	IV/BIW (× 12 doses)	Male/14 Female/16	*Mus musculus*/ iMyc$^{C\alpha}$/Bcl-X_L hybrid (B6 × FVB/N) F_1	Median overall survival[a]	148 days
					Hazard ratio[c]	0.116 (0.057 to 0.236); $p < 0.0001$
					Mean spleen weight + SEM[c,d]	0.63 ± 0.06 g; $p < 0.05$
					Mean IgG2a levels[e]	6.81×10^5 ng/mL; $p < 0.05$
					BW change[f]	73% (Day 203)

BIW = twice weekly; BW = body weight; GEMM = genetically engineered mouse model; Ig = immunoglobulin; IV = intravenous(ly); N/A = not applicable; PCM = plasma cell malignancy; SEM = standard error of the mean.

a Kaplan-Meier estimation; p value of < 0.05 was considered statistically significant.

b Age-matched nontransgenic (B6 × FVB/N) F1 mice were only used for Ig analysis.

c Calculated based on Cox proportional-hazard model, with treatment and sex as covariates; p value of < 0.05 was considered statistically significant.

d Untreated iMycCα/Bcl-XL (n = 29), bortezomib-treated (n = 21), ixazomib-treated (n = 18).

e One-way analysis of variance (ANOVA) compared with untreated controls, adjusted with Dunnett's method to account for multiple comparisons. P values of < 0.05 were considered significant. Age-matched nontransgenic (BL6 × FVB/N) F1 hybrid (n = 9), untreated iMycCα/Bcl-XL (n = 17), bortezomib-treated (n = 18), and ixazomib-treated (n = 13).

f Maximum mean percent body weight change (corresponding study day of measurement); negative values represent a loss in body weight.

2.6.3.2 Primary Pharmacodynamics (continued)

添付資料番号: 4.2.1.1-13

In Vivo Antitumor Activity of Ixazomib in a DP54-Luc Disseminated Mouse Model of a PCM Derived from a GEMM

Group/ Treatment	Dose (mg/kg)	Method of Administration/ Frequency	Sex/ Number per Group[a]	Species/ Strain	Endpoints	Noteworthy Findings
Vehicle (5% HP-β-CD)	N/A	IV/BIW (× 5 doses)	Female/10	*Mus musculus*/ hybrid NOD-SCID bearing disseminated DP54-Luc iMyc$^{C\alpha}$/Bcl-X_L PCM	T/C[b]	N/A
					BW change[c]	-8.1%
					SSSA[d]	1.16 ± 0.04 mm^2
					Photon flux ± SEM (p/s)[e]	$1.22 \times 10^9 \pm 1.20 \times 10^8$
Bortezomib	0.7	IV/BIW (× 5 doses)	Female/10	*Mus musculus*/ hybrid NOD-SCID bearing disseminated DP54-Luc iMyc$^{C\alpha}$/Bcl-X_L PCM	T/C[b]	0.52; $p < 0.05$
					BW change[c]	2.0%
					SSSA[d]	1.17 ± 0.08 mm^2, $p > 0.05$
					Photon flux ± SEM (p/s)[e]	$6.36 \times 10^8 \pm 8.38 \times 10^7$
Ixazomib	11	IV/BIW (× 5 doses)	Female/10	*Mus musculus*/ hybrid NOD-SCID bearing disseminated DP54-Luc iMyc$^{C\alpha}$/Bcl-X_L PCM	T/C[b]	0.36; $p < 0.05$
					BW change[c]	1.9%
					SSSA[d]	0.85 ± 0.16 mm^2, $p < 0.05$
					Photon flux ± SEM (p/s)[e]	$4.33 \times 10^8 \pm 6.47 \times 10^7$
Ixazomib	3	SC/QD (× 15 doses)	Female/10	*Mus musculus*/ hybrid NOD-SCID bearing disseminated DP54-Luc iMyc$^{C\alpha}$/Bcl-X_L PCM	T/C[b]	0.24, $p < 0.05$
					BW change[c]	2.0%
					SSSA[d]	Not measured
					Photon flux ± SEM (p/s)[e]	$2.94 \times 10^8 \pm 4.91 \times 10^7$

BIW = twice weekly; BW = body weight; HP-β-CD = hydroxypropyl-beta-cyclodextrin; GEMM = genetically engineered mouse model; IV = intravenous(ly); N/A = not applicable; NOD-SCID = non-obese diabetic, severe combined immunodeficient; PCM = plasma cell malignancy; p/s = photons/second; QD = once daily; SC = subcutaneous(ly); SEM = standard error of the mean; SSSA = sagittal suture separation area; T/C = treatment over control.

a n = 9 in the ixazomib SC group on Day 20 post-inoculation; n = 9 in the vehicle, bortezomib, and ixazomib SC groups; and n = 5 in the ixazomib IV group on Day 23 post-inoculation.

b T/C values were calculated on Day 20 post-inoculation. Comparisons were made using 2-tailed unpaired t-test; $p < 0.05$ was considered statistically significant.

c Maximum mean percent body weight change; negative values represent a loss in body weight.

d SSSA values were determined using computed tomography (CT) of formalin-fixed mice (postmortem on Day 23 post-inoculation); n = 7 for each group. One-way analysis of variance (ANOVA) was performed on log-transformed data; $p < 0.05$ was considered statistically significant.

e Mean photon flux values were calculated on Day 20 post-inoculation.

2.6.3.2 Primary Pharmacodynamics (continued)

添付資料番号: 4.2.1.1-14

In Vivo Antitumor Activity of Ixazomib in DP54-Luc Intratibial Mouse Model of a PCM Derived from a GEMM

Group/ Treatment	Dose (mg/kg)	Method of Administration/ Frequency	Sex/ Number per Group[a]	Species/ Strain	Endpoints	Noteworthy Findings
Vehicle (5% HP-β-CD)	N/A	IV/BIW (× 6 doses)	Female/8	*Mus musculus*/ athymic nude mice bearing intratibial DP54-Luc iMyc$^{C\alpha}$/Bcl-X_L PCM	T/C[a] BW change[b] Photon flux ± SEM (p/s)[c]	N/A -2.49% (Day 17) $2.28 \times 10^9 \pm 1.18 \times 10^9$
Bortezomib	0.8	IV/BIW (× 6 doses)	Female/8	*Mus musculus*/ athymic nude mice bearing intratibial DP54-Luc iMyc$^{C\alpha}$/Bcl-X_L PCM	T/C[a] BW change[b] Photon flux ± SEM (p/s)[c]	0.02, $p < 0.05$ 5.58% (Day 29) $4.03 \times 10^7 \pm 3.52 \times 10^7$
Ixazomib	13	IV/BIW (× 6 doses)	Female/8	*Mus musculus*/ athymic nude mice bearing intratibial DP54-Luc iMyc$^{C\alpha}$/Bcl-X_L PCM	T/C[a] BW change[b] Photon flux ± SEM (p/s)[c]	0.05, $p < 0.05$ 2.93% (Day 29) $1.19 \times 10^8 \pm 5.14 \times 10^7$

BIW = twice weekly; BW = body weight; GEMM = genetically engineered mouse model; HP-β-CD = hydroxypropyl-beta-cyclodextrin; IV = intravenous(ly); N/A = not applicable; PCM = plasma cell malignancy; p/s = photons/second; SEM = standard error of the mean; T/C = treatment over control.

a T/C = (mean photon flux of a treated group / mean photon flux of the control group). P values were calculated with 2-tailed unpaired t-test with Welch's corrections; $p < 0.05$ was considered statistically significant. T/C values were calculated on Day 29 of treatment.

b Maximum mean percent body weight change (corresponding day of measurement); negative values represent a loss in body weight.

c Mean photon flux and T/C values were calculated on Day 29 of treatment.

2.6.3.3 副次的薬理試験

該当なし。

2.6.3.4 安全性薬理試験

2.6.3.4 Safety Pharmacology

Organ Systems Evaluated	Test System or Species/Strain	Method of Administration	Concentration (μM) or Dose (mg/kg)	Number per Group	Noteworthy Findings	GLP Compliance	添付資料番号
CV	HEK-293 cells stably transfected with the rapidly activating component of the IKr	In vitro	0.00508, 0.0152, 0.0457, 0.137, 0.412, 1.23, 3.70, 11.1, 33.3, and 100	N/A	IC_{50} = 59.6 μmol/L, K_i = 24.9 μmol/L; indicates weak interaction between ixazomib and hERG channel, suggesting potential for prolonging the QT interval.	No	4.2.1.3-1
CV	Dog/beagle	PO	0,[a] 0.021,[b] 0.14,[b] and 0.21[b]	4[c]	No effects at doses up to 0.21 mg/kg[d]	Yes	4.2.1.3-2

CV = cardiovascular; GLP = Good Laboratory Practice; IC_{50} = concentration producing 50% inhibition; IKr = delayed rectifier potassium current; K_i = inhibition dissociation constant; N/A = not applicable; PO = oral(ly).

a Vehicle was 0.5% weight-to-volume ratio (w:v) methylcellulose (MC) solution.

b Administered as ixazomib citrate.

c The same 4 male dogs were used for each dose group. Ixazomib was administered in an ascending dose fashion.

d The results from the toxicokinetic (TK) portion of this study（添付資料番号 4.2.1.3-3）are summarized in Tabulated Summary 2.6.7.3.

Additional information: Electrocardiography (ECG), central nervous system (CNS), and respiratory evaluations were also conducted as part of the GLP- compliant repeat-dose toxicology studies（添付資料番号 4.2.3.2-2; 4.2.3.2-4; 4.2.3.2-5; 4.2.3.2-7; 4.2.3.2-9; 4.2.3.2-11; 4.2.3.2-13; 4.2.3.2-15; 4.2.3.2-16; and 4.2.3.2-20）. The data suggest that ixazomib has a low potential for CV, CNS, or respiratory alterations. The results from these studies are detailed in Tabulated Summaries 2.6.7.7A, 2.6.7.7B, 2.6.7.7C, 2.6.7.7D, 2.6.7.7E, 2.6.7.7F, 2.6.7.7G, 2.6.7.7H, 2.6.7.7I, and 2.6.7.7J.

2.6.3.5 薬力学的薬物相互作用試験

該当なし。