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2.6.5 薬物動態試験概要表

2.6.5.1 薬物動態試験：一覧表

本項では、setmelanotide は RM-493 又は IRC-022493-JM/1 として示す。また、評価に用いた主な製剤は生理食塩液製剤及び mPEG-DSPE（N-[carbonyl-methoxypolyethylene glycol-2000]-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine、平均分子量 2,000）である。なお、setmelanotide はこれらの製剤の成分とは結合していない。

Overview	**Test Article:** Setmelanotide (RM-493)				
Primary Studies Supporting RM-493 Administration in the mPEG-DSPE Formulation in Humans					
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**	**Location in eCTD**
Absorption – Single Dose - Rat					
[^{14}C]-RM-493 ADME Study	Sprague-Dawley Rat	SC and IV Injection	[redacted], [redacted], OH	RM-493-PK-008	4.2.2.2-1
mPEG-DSPE Formulations	Sprague-Dawley Rat	SC Injection	[redacted], [redacted], MI	RM-493-PK-004	4.2.2.2-2
Absorption – Multiple Dose – Rat					
28-Day DRF: Saline Formulation and mPEG-DSPE Formulation (Undiluted)	Sprague-Dawley Rat	SC Injection	[redacted], [redacted], Canada	RM-493-TOX-026	4.2.3.2-1
28-Day DRF: Saline Formulation and mPEG-DSPE Formulation Diluted 1:10 with 5% Mannitol	Sprague-Dawley Rat	SC Injection	[redacted], [redacted], Canada	RM-493-TOX-036	4.2.3.2-2
26-Week Toxicity with a 4-Week Recovery Period: Saline Formulation and mPEG-DSPE Formulation Diluted 1:10 with 5% Mannitol	Sprague-Dawley Rat	SC Injection	[redacted], [redacted], Canada	RM-493-TOX-023	4.2.3.2-3
Fertility and Embryo-fetal Development: mPEG-DSPE Formulation Diluted 1:10 with 5% Mannitol	Sprague-Dawley Rat	SC Injection	[redacted], [redacted], Canada	RM-493-TOX-029	4.2.3.5.1
Pre-/Postnatal Developmental Toxicity: mPEG-DSPE Formulation Diluted 1:10 with 5% Mannitol	Sprague-Dawley Rat	SC Injection	[redacted], [redacted], Canada	RM-493-TOX-033	4.2.3.5.3
DRF in the Juvenile Rat: mPEG-DSPE Formulation Diluted 1:10 with 5% Mannitol	Juvenile Sprague-Dawley Rat	SC Injection	[redacted], [redacted], Canada	RM-493-TOX-034	4.2.3.5.4-1

Overview	Test Article: Setmelanotide (RM-493)				
Primary Studies Supporting RM-493 Administration in the mPEG-DSPE Formulation in Humans					
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**	**Location in eCTD**
7-Week Juvenile Rat with a 4-Week Recovery Period: mPEG-DSPE Formulation Diluted 1:10 with 5% Mannitol	Juvenile Sprague-Dawley Rat	SC Injection	[redacted], Canada	RM-493-TOX-035	4.2.3.5.4-2
Absorption – Multiple Dose – Rabbit					
Tolerability and DRF Embryo-fetal Development Study: mPEG-DSPE Formulation Undiluted and Diluted 1:10 with 5% Mannitol	New Zealand White Rabbit	SC Injection	[redacted], Canada	RM-493-TOX-030	4.2.3.5.2-1
Embryo-fetal Development Study of RM-493 by Subcutaneous Injection in Rabbits: mPEG-DSPE Formulation Diluted 1:10 with 5% Mannitol	New Zealand White Rabbit	SC Injection	[redacted], Canada	RM-493-TOX-031	4.2.3.5.2-2
Absorption – Single Dose – Monkey					
PK Study: mPEG-DSPE and mPEG-DSPE Diluted 1:1 with 5% Mannitol	Cynomolgus Monkey	SC Injection	[redacted], MI	RM-493-PK-006	4.2.2.2-3
PK Crossover Study: Clinical Formulation with and without Preservative	Cynomolgus Monkey	SC Injection	[redacted], MI	RM-493-PK-007	4.2.2.2-4
Absorption – Multiple Dose – Monkey					
28-Day DRF: Saline and Undiluted mPEG-DSPE Formulations	Cynomolgus Monkey	SC Injection	[redacted], Canada	RM-493-TOX-027	4.2.3.2-4
39-Week Toxicity Study with a 4-Week Recovery Period: Saline Formulation and mPEG-DSPE Formulation Diluted 1:10 with 5% Mannitol	Cynomolgus Monkey	SC Injection	[redacted], Canada	RM-493-TOX-024	4.2.3.2-5
Absorption – Multiple Dose – Mouse					
28-Day DRF in Wild Type rasH2 Mice: mPEG-DSPE Diluted 1:10 with 5% Mannitol	Wild type rasH2 Mouse	SC Injection	[redacted], Canada	RM-493-TOX-041	4.2.3.4.2-2
6-Month Carcinogenicity Study in Tg.rasH2 Mice: mPEG-DSPE	Tg.rasH2 Mouse	SC Injection	[redacted], Canada	RM-493-TOX-042	4.2.3.4.2-3
6-Month Carcinogenicity Study in Tg.rasH2 Mice: mPEG-DSPE	Tg.rasH2 Mouse	SC Injection	[redacted], Canada	RM-493-TOX-044	4.2.3.4.2-4
6-Month Carcinogenicity Study in Tg.rasH2 Mice: mPEG-DSPE	Tg.rasH2 Mouse	SC Injection	[redacted], Canada	RM-493-TOX-045	4.2.3.4.2-5

Overview	Test Article: Setmelanotide (RM-493)				
Primary Studies Supporting RM-493 Administration in the mPEG-DSPE Formulation in Humans					
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**	**Location in eCTD**
Distribution					
Binding of RM-493 to Human Plasma Protein	In vitro	NA	[redacted], France	T0106	4.2.2.3-1
Binding to Human, Rat, Mouse and Monkey Plasma Protein	In vitro	NA	[redacted], PA	[redacted]RHYTP4R1	4.2.2.3-2
Binding of RM-493 to Rat and Rabbit Plasma Protein	In vitro	NA	[redacted], PA	[redacted]RHYTP5	4.2.2.3-3
[^{14}C]-RM-493 ADME/QWBA Study	Sprague-Dawley and Long Evans Rat	SC Injection	[redacted], OH	RM-493-PK-008	4.2.2.2-1
[^{14}C]-mPEG-DSPE ADME/QWBA Study	Sprague-Dawley Rat	SC Injection	[redacted], OH	RM-493-PK-013	4.2.2.3-4
Metabolism					
Metabolic Stability on Human Cryopreserved Hepatocytes	In vitro	NA	[redacted], France	T0059	4.2.2.4-1
Assessment of Metabolism on Human Liver Microsomes	In vitro	NA	[redacted], France	T0076	4.2.2.4-2
Stability in Human Fibroblasts	In vitro	NA	[redacted], France	T0115	4.2.2.4-3
Metabolic Stability in Human, Rat, and Monkey Liver and Kidney Microsomes and Cryopreserved Hepatocytes	In vitro	NA	[redacted], PA	[redacted]RHYTP1	4.2.2.4-4
[^{14}C]-RM-493 ADME Study	Sprague-Dawley Rat	IV, SC Injection(s)	[redacted], OH	RM-493-PK-008	4.2.2.2-1
Excretion					
[^{14}C]-RM-493 ADME Study	Sprague-Dawley Rat	IV, SC Injection(s)	[redacted], OH	RM-493-PK-008	4.2.2.2-1

Overview	Test Article: Setmelanotide (RM-493)				
Primary Studies Supporting RM-493 Administration in the mPEG-DSPE Formulation in Humans					
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**	**Location in eCTD**
Pre/Postnatal Development: Excretion During Lactation	Sprague-Dawley Rat	SC Injection	[redacted], [redacted], Canada	RM-493-TOX-033	4.2.3.5.3
Pharmacokinetic Drug Interactions					
In vitro CYP Inhibition Assessment	In vitro	NA	[redacted], France	T0063	4.2.2.6-1
CYP Inhibition in Human Liver Microsomes	In vitro	NA	[redacted], PA	[redacted]RHYTP2_CYP Inhibition	4.2.2.6-2
Time Dependent CYP Inhibition in Human Liver Microsomes	In vitro	NA	[redacted], PA	[redacted]RHYTP2_CYP TDI	4.2.2.6-3
CYP Induction in Human Hepatocytes	In vitro	NA	[redacted], PA	[redacted]RHYTP2_CYP Induction	4.2.2.6-4
AP-BL and BL-AP Permeability Study	In vitro	NA	[redacted], [redacted], WA	791312	4.2.2.6-5
Substrate and Inhibitor Potential for Efflux and Uptake Transporters	In vitro	NA	[redacted], PA	[redacted]RHYTP1	4.2.2.6-6
Substrate and Inhibitor Potential of Setmelanotide for Efflux Transporters MATE1 and MATE2K	In vitro	NA	[redacted], PA	[redacted]RHYTP3	4.2.2.6-7

Overview	Test Article: Setmelanotide (RM-493)				
Supporting PK/ADME Studies					
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**	**Location in eCTD**
Absorption – Single Dose - Rat					
Saline Formulation	Sprague-Dawley Rat	SC Injection	[redacted], [redacted], MI	RM-493-PK-005	4.2.2.7-1
Absorption – Single Dose – Monkey					
Single Dose PK	Rhesus Macaque Monkey	SC Infusion (continuous) and SC Injection	[redacted] Inc, [redacted], MA	BIM-22493-018	4.2.2.7-2
Cardiovascular Response, Tolerability and Efficacy in Obese Monkeys: RM-493 Dissolved in Saline, Various Microparticle Formulations and LY2112688 Infusion	Rhesus Macaque Monkey	SC Injection	[redacted], [redacted], OR	RM-493-PK-002	4.2.2.7-3

Abbreviations: ADME = absorption, distribution, metabolism, and excretion; AP-BL = apical-to-basolateral; BL-AP = basolateral-to-apical; [redacted] = [redacted]; CYP = cytochrome P450; DRF = dose range-finding; eCTD = electronic Common Technical Document; IV = intravenous; MATE = multidrug and toxin extrusion; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; [redacted] = [redacted] PK = pharmacokinetic; QWBA = quantitative whole-body autoradiography; RM-493 = setmelanotide; SC = subcutaneous; Tg.rasH2 = transgenic rasH2.

2.6.5.2 分析法

Species/ Sample Matrix	Analytes	Calibration Range	Type of Assay	Method Utilized	Noteworthy Findings	GLP Compliance	Study Number
Mouse (C57BL/6J) / Plasma	RM-493	5.00 to 2,000 ng/mL	LC-MS/MS	ATM2136/ 4.2.2.1-1	Precision: 3.8 to 8.8% for LLOQ; 1.9 to 4.6% for QCs; Accuracy: 16.0 to 21.8%[a] for LLOQ; -8.0 to 2.0% for QCs	Yes	4003896
Rat[b] (Sprague-Dawley) / Plasma	RM-493	5.00 to 2,000 ng/mL	LC-MS/MS	ATM1697/ 4.2.2.1-2	Precision: 3.6 to 10.0% for LLOQ; 0.9 to 3.6% for QCs; Accuracy: -4.2 to 3.0% for LLOQ; -6.3 to 12.7% for QCs	Yes	03217YZ
Rat (Sprague-Dawley) / Milk	RM-493	5.00 to 2,000 ng/mL	LC-MS/MS	ATM2231/ 4.2.2.1-3	Precision: 6.9 to 10.6% for LLOQ; 0.5 to 4.3% for QCs Accuracy: -16.2 to 4.8% for LLOQ; -6.0 to 5.0% for QCs	Yes	4005518
Rabbit (New Zealand White) / Plasma	RM-493	5.00 to 2,000 ng/mL	LC-MS/MS	ATM1831/ 4.2.2.1-4	Precision: 5.3 to 7.4% for LLOQ; 0.7 to 9.0% for QCs; Accuracy: -1.8 to 5.8% for LLOQ; -2.0 to 7.3% for QCs	Yes	3005991
Monkey[b] (Cynomolgus) / Plasma	RM-493	5.00 to 2,000 ng/mL	LC-MS/MS	ATM1698/ 4.2.2.1-5	Precision: 3.2 to 9.8% for LLOQ; 0.6 to 4.1% for QCs; Accuracy: 0.0 to 4.6% for LLOQ; -5.0 to 7.3% for QCs	Yes	032201V
Mouse (C57BL/6J) / Serum	Anti-RM-493 antibodies	NA	ELISA	AR6450/ 4.2.2.1-6	MRD: 1:20; Stable at RT for 24 hr; Stable for up to 5 freeze thaw cycles; Cut-point of ≥5.4% (1.0% false positive rate); ADA detected in presence of 10,000 ng/mL RM-493	Yes	AR6450
Mouse (C57BL/6J) / Serum	Anti-RM-493 antibodies	NA	ELISA	149-C1103-[redacted] 0057 (AR6450 Addendum 2)/ 4.2.2.1-7	MRD: 1:20; Cut-point of >11.9% (1.0% false positive rate)	Yes	149-C1103-[redacted]0057

Species/ Sample Matrix	Analytes	Calibration Range	Type of Assay	Method Utilized	Noteworthy Findings	GLP Compliance	Study Number
Mouse (C57BL/6J) / Serum	Anti-RM-493 antibodies	NA	ECLIA	TM3186/ 4.2.2.1-8	MRD; 2-fold; Stable at RT for 48 hr; Stable for up to 12 freeze thaw cycles; Cut-point of ≥19.541%; ADA detected in presence of 1,000 ng/mL RM-493	Yes	207255AY VV
Rat (Sprague-Dawley) / Serum	Anti-RM-493 antibodies	NA	ELISA	AR3709/ 4.2.2.1-9	MRD: 1:10; Stable at RT for 24 hr; Stable for up to 5 freeze thaw cycles; Cut-point of ≥30% (0.1% false positive rate); ADA detected in presence of 100 ng/mL RM-493	Yes	AR3709
Rabbit (New Zealand White) / Serum	Anti-RM-493 antibodies	NA	ELISA	AR4135/ 4.2.2.1-10	MRD: 1:50; Stable at RT for 24 hr; Stable for up to 5 freeze thaw cycles; Cut-point of ≥28.6% (0.1% false positive rate); ADA detected in presence of 10 µg/mL RM-493	Yes	AR4135
Monkey (Cynomolgus) / Serum	Anti-RM-493 antibodies	NA	ELISA	AR3708/ 4.2.2.1-11	MRD: 1:10; Stable at RT for 24 hr; Stable for up to 5 freeze thaw cycles; Cut-point of 25% (0.1% false positive rate); ADA detected in presence of 100 ng/mL RM-493	Yes	AR3708

Abbreviations: ADA = anti-drug antibody; ECLIA = electrochemiluminescence immunoassay; ELISA = enzyme linked immunosorbent assay; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantitation; MRD = minimum required dilution; NA = not applicable; QC = quality control; RM-493 = setmelanotide; RT = room temperature.

a) Intra-run bias was outside of acceptance criteria at 21.8% in one run. However, at least two-thirds of the overall LLOQ intra-run data and the overall LLOQ inter-run data met acceptance criteria.

b) Several early formulation studies used non-validated assays that were similar to the validated assays presented herein.

ヒトにおける mPEG-DSPE 製剤による RM-493 の投与を裏付ける主要試験

2.6.5.3 薬物動態：ラットにおける単回投与時の吸収試験

2.6.5.3.1 放射性標識体の ADME 試験（RM-493-PK-008）

Rat PK, Non-GLP	**Test Article:** [^{14}C]-Setmelanotide			
	Location in eCTD: 4.2.2.2-1			
	Study No.: RM-493-PK-008			
Species/Strain:	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**
Gender (M/F)/Number of animals:	9M	9F	9M	9F
Feeding condition:	Fed	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in Saline	RM-493 in Saline	RM-493 in Saline	RM-493 in Saline
Method of Administration:	SC Injection	SC Injection	SC Injection	SC Injection
Dose (mg/kg):	2	2	2	2
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma	Plasma
Analyte:	^{14}C-Equivalents	^{14}C-Equivalents	RM-493	RM-493
Assay:	LSC	LSC	LC-MS/MS	LC-MS/MS
PK parameters:				
AUC_{last} (µg equiv.*hr/g) or (µg*hr/mL)	8.19	7.75	1.42	0.691
AUC_{∞} (µg equiv.*hr /g) or (µg*hr/mL)	9.47[a]	9.03[a]	1.47	NC
C_{max} (µg equiv./g) or (µg/mL)	1.99	1.83	0.674	0.475
t_{max} (hr)	1.00	1.00	0.50	1.00
$t_{1/2}$ (hr)	7.51[a]	7.61[a]	0.759	NC
R^2	0.689	0.713	0.969	NA
AUC_{Extrap} (%)	13.5	14.2	3.21	NA
Bioavailability (%)	76.1	85.2	79	35

Additional Information:
a) Values are approximate due to R^2<0.8.

Abbreviations: ADME = absorption, distribution, metabolism, and excretion; AUC = area under the plasma concentration-time curve; AUC_{∞} = AUC from time 0 to infinity; AUC_{Extrap} = the percentage of AUC_{∞} based on extrapolation; AUC_{last} = AUC from time 0 to the last measurable timepoint; C_{max} = maximum plasma concentration; eCTD = electronic Common Technical Document; F = female; LSC = liquid scintillation counting; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; NA = not applicable; NC = not calculated; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; t_{max} = time to C_{max}.

Rat PK, Non-GLP	**Test Article:** [^{14}C]-Setmelanotide			
	Location in eCTD: 4.2.2.2-1			
	Study No.: RM-493-PK-008			
Species/Strain:	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**
Gender (M/F)/Number of animals:	9M	9F	9M	9F
Feeding condition:	Fed	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in Saline	RM-493 in Saline	RM-493 in Saline	RM-493 in Saline
Method of Administration:	IV Injection	IV Injection	IV Injection	IV Injection
Dose (mg/kg):	1	1	1	1
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma	Plasma
Analyte:	^{14}C-Equivalents	^{14}C-Equivalents	RM-493	RM-493
Assay:	LSC	LSC	LC-MS/MS	LC-MS/MS
PK parameters:				
AUC_{last} (µg equiv.*hr/g) or (µg*hr/mL)	5.38	4.55	0.894	1
AUC_{∞} (µg equiv.*hr /g) or (µg*hr/mL)	6.17[a]	9.99[a]	1.25[b]	1.2
C_0 (µg equiv./g) or (µg/mL)	5.60	5.40	3.01	2.74
$t_{1/2}$ (hr)	5.40[a]	61.9[a]	0.657[b]	0.4
CL (g/hr/kg) or (mL/hr/kg)	162[a]	100[a]	798[b]	832
V_{ss} (g/kg) or (mL/kg)	1,370[a]	6,380[a]	609[b]	412
R^2	0.460	0.950	1.000	1.000
AUC_{Extrap} (%)	12.8	54.5	28.6	16.7

Additional Information:
a) Values are approximations due to R^2 <0.8 or AUC_{Extrap} >20%.
b) Values are approximations due to AUC_{Extrap} >20%.

Abbreviations: AUC = area under the plasma concentration-time curve; AUC_{∞} = AUC from time 0 to infinity; AUC_{Extrap} = the percentage of AUC_{∞} based on extrapolation; AUC_{last} = AUC from time 0 to the last measurable timepoint; C_0 = estimated concentration immediately following IV administration; CL = plasma clearance; eCTD = electronic Common Technical Document; F = female; IV = intravenous; LSC = liquid scintillation counting; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; RM-493 = setmelanotide; $t_{1/2}$ = half-life; V_{ss} = volume of distribution at steady state.

2.6.5.3.2 mPEG-DSPE による単回皮下投与薬物動態試験（RM-493-PK-004）

Rat PK, Non-GLP	**Test Article:** Setmelanotide			
	Location in eCTD: 4.2.2.2-2			
	Study No.: RM-493-PK-004			
Species/Strain:	**Rat/Sprague-Dawley**	**Rat/Sprague-Dawley**	**Rat/Sprague-Dawley**	**Rat/Sprague-Dawley**
Gender (M/F)/Number of animals:	24M	24M	24M	24M
Feeding condition:	Fed	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in mPEG-DSPE (Undiluted)	RM-493 in mPEG-DSPE (Undiluted)	RM-493 in mPEG-DSPE (Undiluted)	RM-493 in mPEG-DSPE Diluted 1:1 with 5% Mannitol
Method of Administration:	SC Injection	SC Injection	SC Injection	SC Injection
Dose (mg/kg):	5	10	30	10
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma	Plasma
Analyte	RM-493	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters:				
t_{max} (hr)	1.00	5.00	3.00	2.00
C_{max} (ng/mL)	431	615	1,230	704
C_{max}/Dose ([ng/mL]/[mg/kg])	86.2	61.5	41.0	70.4
AUC_{last} (ng*hr/mL)	4,192	8,562	35,870	8,915
AUC_{∞} (ng*hr/mL)	4,195	8,571	36,480	8,921
AUC_{∞}/Dose ([ng*hr/mL]/[mg/kg])	839.1	857.1	1,216	892.1
AUC_{Extrap} (%)	0.09	0.10	1.65	0.06
$t_{1/2}$ (hr)	4.56	4.39	8.89	4.14
t_{last} (hr)	48.00	48.00	48.00	48.00
C_{last} (ng/mL)	0.555	1.39	47.1	0.938
CL/F (L/hr/kg)	1.192	1.167	0.8224	1.121
V_z/F (L/kg)	7.834	7.382	10.55	6.690

Abbreviations: AUC = area under the plasma concentration-time curve; AUC_{∞} = AUC from time 0 to infinity; AUC_{∞}/Dose = dose-normalized AUC from time 0 to infinity; AUC_{Extrap} = the percentage of AUC_{∞} based on extrapolation; AUC_{last} = AUC from time 0 to the last measurable timepoint; C_{last} = last measurable plasma concentration; CL/F = apparent plasma clearance; C_{max} = maximum plasma concentration; C_{max}/Dose = dose-normalized C_{max}; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; PK = pharmacokinetic; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; t_{last} = time of last measurable plasma concentration; t_{max} = time to C_{max}; V_z/F = apparent volume of distribution.

2.6.5.4 薬物動態：ラットにおける反復投与時の吸収試験

2.6.5.4.1 28 日間皮下投与用量設定試験（1）（RM-493-TOX-026）

Rat TK, Non-GLP	**Test Article:** Setmelanotide				
	Location in eCTD: 4.2.3.2-1				
	Study No.: RM-493-TOX-026				
Species/Strain:	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**
Gender (M/F)/Number of animals:	12M/12F	12M/12F	12M/12F	12M/12F	12M/12F
Feeding condition:	Fed	Fed	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in Saline	RM-493 in mPEG-DSPE (Undiluted)	RM-493 in mPEG-DSPE (Undiluted)	RM-493 in mPEG-DSPE (Undiluted)	RM-493 in mPEG-DSPE (Undiluted)
Method of Administration:	SC Injection	SC Injection	SC Injection	SC Injection	SC Injection
Dose (mg/kg/day):	30	2	10	20	30
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte:	RM-493	RM-493	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters (Day 1):					
t_{max} (hr)	0.5	2	2	7	8
C_{max} (ng/mL)	2,420	384.5	1,060	1,550	1,655
C_{max}/Dose ([ng/mL]/[mg/kg])	80.65	192.5	106	77.55	55.2
AUC_{0-24} (ng*hr/mL)	6,125	2,410	12,600	21,800	28,200
AUC_{0-24}/Dose ([ng*hr mL]/[mg/kg])	204.25	1,205	1,260	1,089.5	938.5
AUC_{last} (ng*hr/mL)	6,125	2,225	12,600	21,800	28,200
AUC_{last}/Dose ([ng*hr/mL]/[mg/kg])	204.25	1,112.5	1,260	1,089.5	938.5
$t_{1/2}$ (hr)	4.305	2.35	3.655	6.46	13.7

Rat TK, Non-GLP	**Test Article:** Setmelanotide				
	Location in eCTD: 4.2.3.2-1				
	Study No.: RM-493-TOX-026				
Species/Strain:	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**
Vehicle/Formulation:	RM-493 in Saline	RM-493 in mPEG-DSPE (Undiluted)	RM-493 in mPEG-DSPE (Undiluted)	RM-493 in mPEG-DSPE (Undiluted)	RM-493 in mPEG-DSPE (Undiluted)
Dose (mg/kg/day):	30	2	10	20	30
PK parameters (Day 22/23, except for 30 mg/kg mPEG-DSPE, which is Day 15/16):					
t_{max} (hr)	2	2	3	4	3
C_{max} (ng/mL)	8,555	588	2,095	2,500	3,510
C_{max}/Dose ([ng/mL]/[mg/kg])	285.5	293.5	209.5	125	117
$AUC_{0-\tau}$ (ng*hr/mL)	38,550	3,750	17,900	29,600	39,100
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	1,285	1,875	1,790	1,480	1,305
AUC_{last} (ng*hr/mL)	38,700	3,710	17,900	30,550	40,500
AUC_{last}/Dose ([ng*hr/mL]/[mg/kg])	1,290	1,855	1,790	1,530	1,350
C_{avg} (ng/mL)	1,610	156	745.5	1,230	1,630
$t_{1/2}$ (hr)	3.36	1.925	2.715	3.89	4.395
CL_{ss}/F (L/hr/kg)	0.808	0.541	0.564	0.68	0.7695
V_z/F (L/kg)	3.705	1.475	2.185	3.81	4.905
R_{ac}[a]	8.07	1.575	1.445	1.415	1.425
Additional Information: Due to clinical observations noted, dosing for animals in the 30 mg/kg/day mPEG-DSPE group was discontinued on Day 14/15 (main study animals) and Day 15/16 (TK animals). Due to clinical observations noted, Main Study animals in all other dose groups were sent to necropsy on Day 23/22. TK animals in these groups received a final dose on Day 23/22, followed by TK and anti-drug antibody evaluations. a) R_{ac} = [$AUC_{0-\tau}$ (Day 23/22/16/15)]/ [AUC_{0-24} (Day 1)].					

Abbreviations: AUC = area under the plasma concentration-time curve; AUC_{0-24} = AUC from time 0 to 24 hours postdose; $AUC_{0-\tau}$ = AUC during a time interval; $AUC_{0-\tau}$/Dose = dose-normalized AUC during a time interval; AUC_{last} = AUC from time 0 to the last measurable timepoint; AUC_{last}/Dose = dose-normalized AUC from time 0 to last measurable timepoint; C_{avg} = average plasma concentration; CL_{ss}/F = apparent plasma clearance at steady state; C_{max} = maximum plasma concentration; C_{max}/Dose = dose-normalized C_{max}; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; PK = pharmacokinetic; R_{ac} = systemic accumulation ratio; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; TK = toxicokinetic; t_{max} = time to C_{max}; V_z/F = apparent volume of distribution.

2.6.5.4.2 28 日間皮下投与用量設定試験（2）（RM-493-TOX-036）

Rat TK, Non-GLP	**Test Article:** Setmelanotide							
	Location in eCTD: 4.2.3.2-2							
	Study No.: RM-493-TOX-036							
Species/Strain:	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**
Gender (M/F)/Number of animals:	12M/12F	12M/12F	12M/12F	12M/12F	12M/12F	12M/12F	12M/12F	12M/12F
Feeding condition:	Fed	Fed	Fed	Fed	Fed	Fed	Fed	Fed
Vehicle/ Formulation:	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in Saline	RM-493 in Saline	RM-493 in Saline	RM-493 in Saline	RM-493 in Saline
Method of Administration:	SC Injection	SC Injection	SC Injection	SC Injection	SC Injection	SC Injection	SC Injection	SC Injection
Dose (mg/kg/day):	1	3	5	2.5	5	7.5	15	30 (BID; 15 mg/kg/dose)
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte:	RM-493	RM-493	RM-493	RM-493	RM-493	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters (Day 1):								
t_{max} (hr)	0.50	2.00	2.00	0.50	0.50	0.50	0.50	0.50
C_{max} (ng/mL)	331	620	880	631	1,060	1,260	1,710	2,280
C_{max}/Dose ([ng/mL]/[mg/kg])	331	207	176	252	213	169	114	152
$AUC_{0-\tau}$ (ng*hr/mL)	974	2,720	4,340	1,110	2,140	2,700	5,080	4,970
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	974	907	867	443	428	359	339	331
AUC_{last} (ng*hr/mL)	972	2,660	4,180	1,110	2,130	2,700	5,050	4,970
AUC_{last}/Dose ([ng*hr/mL]/[mg/kg])	972	888	835	442	427	359	337	331
$t_{1/2}$ (hr)	NC	NC	NC	NC	NC	NC	NC	NC

Rat TK, Non-GLP	**Test Article:** Setmelanotide							
	Location in eCTD: 4.2.3.2-2							
	Study No.: RM-493-TOX-036							
Species/Strain:	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**
Vehicle/ Formulation:	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in Saline	RM-493 in Saline	RM-493 in Saline	RM-493 in Saline	RM-493 in Saline
Dose (mg/kg/day):	1	3	5	2.5	5	7.5	15	30 (BID; 15 mg/kg/dose)
PK parameters (Day 28):								
t_{max} (hr)	0.50	2.00	2.00	2.00	2.00	2.00	2.00	2.00
C_{max} (ng/mL)	289	700	1,270	719	1,550	2,960	4,950	4,840
C_{max}/Dose ([ng/mL]/[mg/kg])	289	233	253	288	310	394	330	323
$AUC_{0-\tau}$ (ng*hr/mL)	1,360	4,550	7,790	2,950	6,900	11,800	24,500	22,000
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	1,360	1,520	1,560	1,180	1,380	1,570	1,630	1,470
AUC_{last} (ng*hr/mL)	1,330	4,390	7,790	2,930	6,850	11,800	24,500	22,000
AUC_{last}/Dose ([ng*hr/mL]/[mg/kg])	1,330	1,460	1,560	1,170	1,370	1,570	1,630	1,470
C_{avg} (ng/mL)	56.5	190	325	123	288	490	1,020	1,830
$t_{1/2}$ (hr)	1.42	1.89	2.77	1.25	1.20	2.28	2.32	1.47
CL_{ss}/F (L/hr/kg)	0.737	0.659	0.642	0.847	0.724	0.638	0.613	0.683
V_z/F (L/kg)	1.51	1.79	2.57	1.52	1.25	2.10	2.06	1.45
R_{ac}[a]	1.39	1.67	1.80	2.66	3.23	4.36	4.81	4.42
Additional Information: a) R_{ac} = [$AUC_{0-\tau}$ (Day 28)] / [$AUC_{0-\tau}$ (Day 1)].								

Abbreviations: AUC = area under the plasma concentration-time curve; $AUC_{0-\tau}$ = AUC during a time interval; $AUC_{0-\tau}$/Dose = dose-normalized AUC during a time interval; AUC_{last} = AUC from time 0 to the last measurable timepoint; AUC_{last}/Dose = dose-normalized AUC from time 0 to last measurable timepoint; BID = twice daily; C_{avg} = average plasma concentration; CL_{ss}/F = apparent plasma clearance at steady state; C_{max} = maximum plasma concentration; C_{max}/Dose = dose-normalized C_{max}; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; NC = not calculated; PK = pharmacokinetic; R_{ac} = systemic accumulation ratio; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; TK = toxicokinetic; t_{max} = time to C_{max}; V_z/F = apparent volume of distribution.

2.6.5.4.3 26 週間皮下投与毒性試験及び 4 週間回復性試験（RM-493-TOX-023）

Rat TK, GLP	**Test Article:** Setmelanotide				
	Location in eCTD: 4.2.3.2-3				
	Study No.: RM-493-TOX-023				
Species/Strain:	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**
Gender (M/F)/Number of animals:	12M/12F	12M/12F	12M/12F	12M/12F	12M/12F
Feeding condition:	Fed	Fed	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in Saline	RM-493 in Saline	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol
Method of Administration:	SC Injection	SC Injection	SC Injection	SC Injection	SC Injection
Dose (mg/kg/day):	3	15	0.5	1.5	3.0
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte:	RM-493	RM-493	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters (Day 1):					
t_{max} (hr)	0.500	0.500	0.500	0.500	0.500
C_{max} (ng/mL)	548	1,390	121	313	488
C_{max}/Dose ([ng/mL]/[mg/kg])	183	92.5	242	209	163
$AUC_{0-\tau}$ (ng*hr/mL)	1,120	4,710	340	1,090	2,120
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	375	314	680	729	708
$t_{1/2}$ (hr)	NE	0.948	NE	1.35	1.62
PK parameters (Day 90):					
t_{max} (hr)	0.500	2.00	2.00	2.00	2.00
C_{max} (ng/mL)	805	5,570	145	561	1,010
C_{max}/Dose ([ng/mL]/[mg/kg])	268	372	290	374	337
$AUC_{0-\tau}$ (ng*hr/mL)	3,950	26,600	547	2,650	5,820
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	1,320	1,770	1,090	1,770	1,940
$t_{1/2}$ (hr)	1.53	2.25	NE	2.46	1.91

Rat TK, GLP	**Test Article:** Setmelanotide				
	Location in eCTD: 4.2.3.2-3				
	Study No.: RM-493-TOX-023				
Species/Strain:	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**
Vehicle/Formulation:	RM-493 in Saline	RM-493 in Saline	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol
Dose (mg/kg/day):	3	15	0.5	1.5	3.0
PK parameters (Day 180):					
t_{max} (hr)	0.500	4.00	0.500	2.00	2.00
C_{max} (ng/mL)	849	4,350	130	365	675
C_{max}/Dose ([ng/mL]/[mg/kg])	283	290	261	244	225
$AUC_{0-\tau}$ (ng*hr/mL)	4,100	28,400	809	2,120	5,100
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	1,370	1,900	1,620	1,410	1,700
$t_{1/2}$ (hr)	NE	3.58	NE	NE	6.32
CL_{ss}/F (L/hr/kg)	0.732	0.527	0.618	0.709	0.588
V_z/F (L/kg)	NE	2.73	NE	NE	5.36
R_{ac}[a]	3.65	6.04	2.38	1.93	2.40

Additional Information:
a) R_{ac} = [$AUC_{0-\tau}$ (Day 180)] / [$AUC_{0-\tau}$ (Day 1)].

Abbreviations: AUC = area under the plasma concentration-time curve; $AUC_{0-\tau}$ = AUC during a time interval; $AUC_{0-\tau}$/Dose = dose-normalized AUC during a time interval; CL_{ss}/F = apparent plasma clearance at steady state; C_{max} = maximum plasma concentration; C_{max}/Dose = dose-normalized C_{max}; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; NE = not estimable; PK = pharmacokinetic; R_{ac} = systemic accumulation ratio; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; TK = toxicokinetic; t_{max} = time to C_{max}; V_z/F = apparent volume of distribution.

2.6.5.4.4 雌ラットを用いた受胎能及び胚・胎児発生に関する試験（RM-493-TOX-029）

Rat TK, GLP	**Test Article:** Setmelanotide		
	Location in eCTD: 4.2.3.5.1		
	Study No.: RM-493-TOX-029		
Species/Strain:	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**
Gender (M/F)/Number of animals:	9F	9F	9F
Feeding condition:	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol
Method of Administration:	SC Injection	SC Injection	SC Injection
Dose (mg/kg/day):	0.5	3.0	5.0
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma
Analyte:	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters (Day 1):			
t_{max} (hr)	0.500	2.00	0.500
C_{max} (ng/mL)	130	470	445
C_{max}/Dose ([ng/mL]/[mg/kg])	261	157	88.9
$AUC_{0-\tau}$ (ng*hr/mL)	396	1,870	3,190
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	792	623	638
$t_{1/2}$ (hr)	NE	2.26	2.15
PK parameters (GD 17):			
t_{max} (hr)	0.500	2.00	0.500
C_{max} (ng/mL)	147	608	1,280
C_{max}/Dose ([ng/mL]/[mg/kg])	294	203	257
$AUC_{0-\tau}$ (ng*hr/mL)	388	3,150	6,450
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	775	1,050	1,290
$t_{1/2}$ (hr)	1.25	2.10	2.50
CL_{ss}/F (L/hr/kg)	1.29	0.953	0.775
V_z/F (L/kg)	2.33	2.88	2.79
R_{ac}[a]	0.979	1.68	2.02

Additional Information:
a. R_{ac} = [$AUC_{0-\tau}$ (GD 17)] / [$AUC_{0-\tau}$ (Day 1)].

Abbreviations: AUC = area under the plasma concentration-time curve; $AUC_{0-\tau}$ = AUC during a time interval; $AUC_{0-\tau}$/Dose = dose-normalized AUC during a time interval; CL_{ss}/F = apparent

plasma clearance at steady state; C_{max} = maximum plasma concentration; C_{max}/Dose = dose-normalized C_{max}; eCTD = electronic Common Technical Document; F = female; GD = gestation day; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; NE = not estimable; PK = pharmacokinetic; R_{ac} = systemic accumulation ratio; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; TK = toxicokinetic; t_{max} = time to C_{max}; V_z/F = apparent volume of distribution.

2.6.5.4.5 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（RM-493-TOX-033）

Rat TK, GLP	**Test Article:** Setmelanotide		
	Location in eCTD: 4.2.3.5.3		
	Study No.: RM-493-TOX-033		
Species/Strain:	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**
Gender (M/F)/Number of animals:	8F	8F	8F
Feeding condition:	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol
Method of Administration:	SC Injection	SC Injection	SC Injection
Dose (mg/kg/day):	0.5	3.0	5.0
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma
Analyte:	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters (Day 20 PC, F_0 Generation):			
t_{max} (hr)	0.500	2.00	2.00
C_{max} (ng/mL)	101	541	744
C_{max}/Dose ([ng/mL]/[mg/kg])	203	180	149
AUC_{last} (ng*hr/mL)	231	2,050	4,140
AUC_{last}/Dose ([ng*hr/mL]/[mg/kg])	462	682	828
$AUC_{0-\tau}$ (ng*hr/mL)	NE	NE	4,370
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	NE	NE	874
$t_{1/2}$ (hr)	NE	NE	2.80
CL_{ss}/F (L/hr/kg)	NE	NE	1.14
V_z/F (L/kg)	NE	NE	4.62

Rat TK, GLP	**Test Article:** Setmelanotide		
	Location in eCTD: 4.2.3.5.3		
	Study No.: RM-493-TOX-033		
Species/Strain:	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**
Vehicle/Formulation:	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol
Dose (mg/kg/day):	0.5	3.0	5.0
PK parameters (LD 10, F_0 Generation):			
t_{max} (hr)	0.500	4.00	2.00
C_{max} (ng/mL)	71.2	427	990
C_{max}/Dose ([ng/mL]/[mg/kg])	142	142	198
AUC_{last} (ng*hr/mL)	247	2,340	4,340
AUC_{last}/Dose ([ng*hr/mL]/[mg/kg])	495	781	869
$AUC_{0-\tau}$ (ng*hr/mL)	NE	NE	4,420
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	NE	NE	884
$t_{1/2}$ (hr)	NE	NE	1.89
CL_{ss}/F (L/hr/kg)	NE	NE	1.13
V_z/F (L/kg)	NE	NE	3.08
Additional Information: F_1 Generation- no measurable concentrations of RM-493 in plasma were observed for pups; Measurable RM-493 concentrations in milk were observed for the 0.5, 3 and 5 mg/kg/day groups. Mean RM-493 concentrations in milk ranged from 12.0 to 106 ng/mL.			

Abbreviations: AUC = area under the plasma concentration-time curve; $AUC_{0-\tau}$ = AUC during a time interval; $AUC_{0-\tau}$/Dose = dose-normalized AUC during a time interval; AUC_{last} = AUC from time 0 to the last measurable timepoint; AUC_{last}/Dose = dose-normalized AUC from time 0 to the last measurable timepoint; CL_{ss}/F = apparent plasma clearance at steady state; C_{max} = maximum plasma concentration; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LD = lactation day; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; NE = not estimable; PC = post-coitum; PK = pharmacokinetic; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; TK = toxicokinetic; t_{max} = time to C_{max}; V_z/F = apparent volume of distribution.

2.6.5.4.6 幼若ラットを用いた用量設定試験（RM-493-TOX-034）

Juvenile Rat TK, Non-GLP	**Test Article:** Setmelanotide			
	Location in eCTD: 4.2.3.5.4-1			
	Study No.: RM-493-TOX-034			
Species/Strain:	**Rat/ Sprague-Dawley (Juvenile)**	**Rat/ Sprague-Dawley (Juvenile)**	**Rat/ Sprague-Dawley (Juvenile)**	**Rat/ Sprague-Dawley (Juvenile)**
Gender (M/F)/Number of animals:	6M/6F	6M/6F	6M/6F	6M/6F
Feeding condition:	Fed	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in Saline
Method of Administration:	SC Injection	SC Injection	SC Injection	SC Injection
Dose (mg/kg/day):	1	2.5	5	15
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma	Plasma
Analyte:	RM-493	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters (Day 7 pp):				
t_{max} (hr)	2.00	2.00	2.00	2.00
C_{max} (ng/mL)	165	455	1,060	1,690
C_{max}/Dose ([ng/mL]/[mg/kg])	165	182	212	113
$AUC_{0-\tau}$ (ng*hr/mL)	885	1,870	5,710	11,300
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	885	748	1,140	751
AUC_{last} (ng*hr/mL)	380	997	2,450	4,100
AUC_{last}/Dose ([ng*hr/mL]/[mg/kg])	380	399	490	273
$t_{1/2}$ (hr)	NC	NC	NC	NC

Juvenile Rat TK, Non-GLP	**Test Article:** Setmelanotide			
	Location in eCTD: 4.2.3.5.4-1			
	Study No.: RM-493-TOX-034			
Species/Strain:	**Rat/ Sprague-Dawley (Juvenile)**	**Rat/ Sprague-Dawley (Juvenile)**	**Rat/ Sprague-Dawley (Juvenile)**	**Rat/ Sprague-Dawley (Juvenile)**
Vehicle/Formulation:	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in Saline
Dose (mg/kg/day):	1	2.5	5	15
PK parameters (Day 34 pp):				
t_{max} (hr)	1.00	1.00	1.00	1.00
C_{max} (ng/mL)	406	824	2,300	5,300
C_{max}/Dose ([ng/mL]/[mg/kg])	406	330	459	353
$AUC_{0-\tau}$ (ng*hr/mL)	745	1,920	5,350	11,200
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	745	768	1,070	744
AUC_{last} (ng*hr/mL)	740	1,910	5,320	11,100
AUC_{last}/Dose ([ng*hr/mL]/[mg/kg])	740	763	1,060	743
$t_{1/2}$ (hr)	0.790	1.02	1.23	1.04
CL_{ss}/F (L/hr/kg)	1.34	1.30	0.935	1.34
V_z/F (L/kg)	1.53	1.92	1.66	2.01
R_{ac}[a]	0.842	1.03	0.937	0.991

Additional Information:

a) R_{ac} = [$AUC_{0-\tau}$ (Day 34 pp)] / [$AUC_{0-\tau}$ (Day 7 pp)].

Abbreviations: AUC = area under the plasma concentration-time curve; $AUC_{0-\tau}$ = AUC during a time interval; $AUC_{0-\tau}$/Dose = dose-normalized AUC during a time interval; AUC_{last} = AUC from time 0 to the last measurable timepoint; AUC_{last}/Dose = dose-normalized AUC from time 0 to the last measurable timepoint; CL_{ss}/F = apparent plasma clearance at steady state; C_{max} = maximum plasma concentration; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; NC = not calculated; PK = pharmacokinetic; pp = post-partum; R_{ac} = systemic accumulation ratio; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; TK = toxicokinetic; t_{max} = time to C_{max}; V_z/F = apparent volume of distribution.

2.6.5.4.7 幼若ラットを用いた 7 週間皮下投与毒性試験及び 4 週間回復性試験（RM-493-TOX-035）

Juvenile Rat TK, GLP	**Test Article:** Setmelanotide			
	Location in eCTD: 4.2.3.5.4-2			
	Study No.: RM-493-TOX-035			
Species/Strain:	**Rat/ Sprague-Dawley (Juvenile)**	**Rat/ Sprague-Dawley (Juvenile)**	**Rat/ Sprague-Dawley (Juvenile)**	**Rat/ Sprague-Dawley (Juvenile)**
Gender (M/F)/Number of animals:	36M/36F	36M/36F	36M/36F	36M/36F
Feeding condition:	Fed	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in Saline
Method of Administration:	SC Injection	SC Injection	SC Injection	SC Injection
Dose (mg/kg/day):	0.5	1.5	3	15
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma	Plasma
Analyte:	RM-493	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters (Day 7 pp):				
t_{max} (hr)	1.00	1.00	1.00	1.00
C_{max} (ng/mL)	179	510	861	4,540
C_{max}/Dose ([ng/mL]/[mg/kg])	359	340	287	302
$AUC_{0-\tau}$ (ng*hr/mL)	429	1,580	2,920	9,210
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	859	1,050	972	614
$t_{1/2}$ (hr)	NE	1.21	1.52	1.42
CL_{ss}/F (L/hr/kg)	NE	NE	NE	NE
V_z/F (L/kg)	NE	NE	NE	NE

Juvenile Rat TK, GLP	**Test Article:** Setmelanotide			
	Location in eCTD: 4.2.3.5.4-2			
	Study No.: RM-493-TOX-035			
Species/Strain:	**Rat/ Sprague-Dawley (Juvenile)**	**Rat/ Sprague-Dawley (Juvenile)**	**Rat/ Sprague-Dawley (Juvenile)**	**Rat/ Sprague-Dawley (Juvenile)**
Vehicle/Formulation:	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in Saline
Dose (mg/kg/day):	0.5	1.5	3	15
PK parameters (Day 55 pp):				
t_{max} (hr)	1.00	2.00	1.00	4.00
C_{max} (ng/mL)	158	421	805	3,560
C_{max}/Dose ([ng/mL]/[mg/kg])	317	281	268	237
$AUC_{0-\tau}$ (ng*hr/mL)	452	1,760	3,840	18,800
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	904	1,170	1,280	1,250
$t_{1/2}$ (hr)	0.845	NE	1.50	NE
CL_{ss}/F (L/hr/kg)	1.11	0.854	0.781	0.798
V_z/F (L/kg)	1.35	NE	1.69	NE
R_{ac}[a]	1.05	1.11	1.32	2.04

Additional Information:

a) R_{ac} = [$AUC_{0-\tau}$ (Day 55 pp)] / [$AUC_{0-\tau}$ (Day 7 pp)].

Abbreviations: AUC = area under the plasma concentration-time curve; $AUC_{0-\tau}$ = AUC during a time interval; $AUC_{0-\tau}$/Dose = dose-normalized AUC during a time interval; CL_{ss}/F = apparent plasma clearance at steady state; C_{max} = maximum plasma concentration; C_{max}/Dose = dose-normalized C_{max}; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; NE = not estimable; PK = pharmacokinetic; pp = post-partum; R_{ac} = systemic accumulation ratio; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; TK = toxicokinetic; t_{max} = time to C_{max}; V_z/F = apparent volume of distribution.

2.6.5.5 薬物動態：ウサギにおける反復投与時の吸収試験

2.6.5.5.1 忍容性及び胚・胎児発生用量設定試験（RM-493-TOX-030）

Rabbit TK, Non-GLP	**Test Article:** Setmelanotide				
	Location in eCTD: 4.2.3.5.2-1				
	Study No.: RM-493-TOX-030				
Species/Strain:	**Rabbit/ New Zealand White**	**Rabbit/ New Zealand White**	**Rabbit/ New Zealand White**	**Rabbit/ New Zealand White**	**Rabbit/ New Zealand White**
Gender (M/F)/Number of animals:	6F	6F	6F	6F	6F
Feeding condition:	Fed	Fed	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol
Method of Administration:	SC Injection	SC Injection	SC Injection	SC Injection	SC Injection
Dose (mg/kg/day):	0.05	0.5	3	10	20
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte:	RM-493	RM-493	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Mean PK parameters (Day 7 pc, Sub-Group A):					
t_{max} (hr) (Median)	1.00	2.00	4.00	4.00	12.00
C_{max} (ng/mL)	78.2	570	2,590	8,570	7,100
C_{max}/Dose ([ng/mL]/[mg/kg])	1,560	1,140	864	857	355
$AUC_{0-\tau}$ (ng*hr/mL)	155	2,270	22,100	76,800	106,000
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	3,100	4,540	7,380	7,680	5,290
$t_{1/2}$ (hr)	NC	1.15	2.22	2.14	NC

Rabbit TK, Non-GLP	**Test Article:** Setmelanotide				
	Location in eCTD: 4.2.3.5.2-1				
	Study No.: RM-493-TOX-030				
Species/Strain:	**Rabbit/ New Zealand White**	**Rabbit/ New Zealand White**	**Rabbit/ New Zealand White**	**Rabbit/ New Zealand White**	**Rabbit/ New Zealand White**
Vehicle/Formulation:	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol
Dose (mg/kg/day):	0.05	0.5	3	10	20
Mean PK parameters (Day 13 pc, Sub-Group A):					
t_{max} (hr) (Median)	1.00	1.00	2.00	2.00	4.00
C_{max} (ng/mL)	79.3	532	2,560	8,010	12,900
C_{max}/Dose ([ng/mL]/[mg/kg])	1,590	1,060	854	801	645
$AUC_{0-\tau}$ (ng*hr/mL)	194	1,830	15,800	53,900	135,000
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	3,890	3,660	5,280	5,390	6,740
$t_{1/2}$ (hr)	1.08	0.885	2.62	1.92	2.93
CL_{ss}/F (L/hr/kg)	0.271	0.282	0.194	0.188	0.151
V_z/F (L/kg)	0.323	0.351	0.733	0.505	0.645
R_{ac}[a]	1.25	0.807	0.714	0.725	1.27
Mean PK parameters (Day 13 pc, Sub-Group B):					
t_{max} (hr) (Median)	1.00	2.00	4.00	6.00	8.00
C_{max} (ng/mL)	108	370	2,440	8,840	8,640
C_{max}/Dose ([ng/mL]/[mg/kg])	2,160	741	813	884	432
$AUC_{0-\tau}$ (ng*hr/mL)	199	2,250	22,100	104,000	131,000
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	3,970	4,500	7,360	10,400	6,540
$t_{1/2}$ (hr)	NC	1.37	2.27	2.43	NC
CL_{ss}/F (L/hr/kg)	0.282	0.224	0.137	0.0992	0.159
V_z/F (L/kg)	NC	0.442	0.449	0.307	NC
R_{ac}	NC	NC	NC	NC	NC

Rabbit TK, Non-GLP	**Test Article:** Setmelanotide				
	Location in eCTD: 4.2.3.5.2-1				
	Study No.: RM-493-TOX-030				
Species/Strain:	**Rabbit/ New Zealand White**	**Rabbit/ New Zealand White**	**Rabbit/ New Zealand White**	**Rabbit/ New Zealand White**	**Rabbit/ New Zealand White**
Vehicle/Formulation:	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol
Dose (mg/kg/day):	0.05	0.5	3	10	20
Mean PK parameters (Day 19 pc, Sub-Group B):					
t_{max} (hr) (Median)	1.00	1.50	2.00	4.00	6.00
C_{max} (ng/mL)	70.5	409	2,930	8,770	8,660
C_{max}/Dose ([ng/mL]/[mg/kg])	1,410	818	977	877	433
$AUC_{0-\tau}$ (ng*hr/mL)	162	1,380	12,400	61,600	104,000
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	3,240	2,760	4,130	6,160	5,210
$t_{1/2}$ (hr)	NC	1.78	1.18	1.70	NC
CL_{ss}/F (L/hr/kg)	0.318	0.365	0.260	0.177	0.193
V_z/F (L/kg)	NC	1.02	0.441	0.409	NC
R_{ac} [b]	0.877	0.580	0.564	0.580	0.827

Additional Information: 3 animals per group were dosed from Days 7 to 13 pc, and the other 3 from days 13 to 19 pc.

a) The accumulation ratio for Day 13 pc, calculated as $AUC_{0-\tau}$ (Day 13 pc) / $AUC_{0-\tau}$ (Day 7 pc) was reported for informational purposes.

b) R_{ac} = [$AUC_{0-\tau}$ (Day 19 pc)] / [$AUC_{0-\tau}$ (Day 13 pc)].

Abbreviations: AUC = area under the plasma concentration-time curve; $AUC_{0-\tau}$ = AUC during a time interval; $AUC_{0-\tau}$/Dose = dose-normalized AUC during a time interval; CL_{ss}/F = apparent plasma clearance at steady state; C_{max} = maximum plasma concentration; C_{max}/Dose = dose-normalized C_{max}; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; NC = not calculated; pc = post-coitum; PK = pharmacokinetic; R_{ac} = systemic accumulation ratio; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; TK = toxicokinetic; t_{max} = time to C_{max}; V_z/F = apparent volume of distribution.

2.6.5.5.2 胚・胎児発生に関する試験（RM-493-TOX-031）

Rabbit TK, GLP	**Test Article:** Setmelanotide				
	Location in eCTD: 4.2.3.5.2-2				
	Study No.: RM-493-TOX-031				
Species/Strain:	**Rabbit/ New Zealand White**	**Rabbit/ New Zealand White**	**Rabbit/ New Zealand White**	**Rabbit/ New Zealand White**	**Rabbit/ New Zealand White**
Gender (M/F)/Number of animals:	20F	20F	22F	20F	22F
Feeding condition:	Fed	Fed	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol
Method of Administration:	SC Injection	SC Injection	SC Injection	SC Injection	SC Injection
Dose (mg/kg/day):	0.05	0.05	0.1	0.2	0.2
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte:	RM-493	RM-493	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Mean PK parameters (GD 7/13):	**GD 7**	**GD 13**	**GD 13**	**GD 7**	**GD 13**
t_{max} (hr)	1.00	1.00	1.00	2.00	1.00
C_{max} (ng/mL)	60.7	88.8	152	244	319
C_{max}/Dose ([ng/mL]/[mg/kg])	1,210	1,780	1,520	1,220	1,590
$AUC_{0-\tau}$ (ng*hr/mL)	149	223	524	958	1,160
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	2,980	4,450	5,240	4,790	5,800
$t_{1/2}$ (hr)	NE	NE	1.02	NE	NE
Mean PK parameters (GD 13/19):	**GD 13**	**GD 19**	**GD 19**	**GD 13**	**GD 19**
t_{max} (hr)	1.00	2.00	1.00	1.00	1.00
C_{max} (ng/mL)	77.9	53.0	139	212	242
C_{max}/Dose ([ng/mL]/[mg/kg])	1,560	1,060	1,390	1,060	1,210
$AUC_{0-\tau}$ (ng*hr/mL)	214	206	436	941	862
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	4,280	4,120	4,360	4,710	4,310
$t_{1/2}$ (hr)	NE	NE	1.12	1.77	NE
R_{ac}[a]	1.44	0.926	0.832	0.983	0.743

Additional Information: 3 animals per group were dosed from GD 7 to 13, and the other 3 from GD 13 to 19.
a) R_{ac} = [$AUC_{0-\tau}$ (last dose)] / [$AUC_{0-\tau}$ (first dose)].

Abbreviations: AUC = area under the plasma concentration-time curve; $AUC_{0-\tau}$ = AUC during a time interval; $AUC_{0-\tau}$/Dose = dose-normalized AUC during a time interval; C_{max} = maximum

plasma concentration; eCTD = electronic Common Technical Document; F = female; GD = gestational day; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; NE = not estimable; PK = pharmacokinetic; R_{ac} = systemic accumulation ratio; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; TK = toxicokinetic; t_{max} = time to C_{max}.

2.6.5.6 薬物動態：サルにおける単回投与時の吸収試験

2.6.5.6.1 カニクイザルを用いた単回皮下投与薬物動態試験（RM-493-PK-006）

Monkey PK, Non-GLP	**Test Article:** Setmelanotide				
	Location in eCTD: 4.2.2.2-3				
	Study No.: RM-493-PK-006				
Species/Strain:	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**
Gender (M/F)/Number of animals:	6M	6M	6M	6M	6M
Feeding condition:	Fed	Fed	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in mPEG-DSPE	RM-493 in mPEG-DSPE	RM-493 in mPEG-DSPE	RM-493 in mPEG-DSPE	RM-493 in mPEG-DSPE Diluted 1:1 with 5% Mannitol
Method of Administration:	SC Injection	SC Injection	SC Injection	SC Injection	SC Injection
Dose (mg/kg):	0.5	1	5	10	5
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte:	RM-493	RM-493	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Mean PK parameters:					
t_{max} (hr)	8.00	9.00	11.33	11.33	9.00
C_{max} (ng/mL)	344	687	2,650	6,420	3,220
C_{max}/Dose ([ng/mL]/[mg/kg])	689	687	530	642	643
AUC_{last} (ng*hr/mL)	5,045	10,790	51,370	115,100	49,810
AUC_{last}/Dose ([ng*hr/mL]/[mg/kg])	10,090	10,790	10,270	11,510	9,962
t_{last} (hr)	48.00	48.00	48.00	48.00	48.00
C_{last} (ng/mL)	1.99	3.78	22.3	64.0	15.5
AUC_{∞} (ng*hr/mL)	5,252	9,509	59,220 (N=1)	131,000 (N=1)	50,680
AUC_{∞}/Dose ([ng*hr/mL]/[mg/kg])	10,500	9,509	11,840 (N=1)	13,100 (N=1)	10,140
AUC_{Extrap} (%)	0.26	0.23	0.38 (N=1)	0.22 (N=1)	0.25
λ_z (1/hr)	0.1365	0.1364	0.1309 (N=1)	0.1435 (N=1)	0.140
$t_{1/2}$ (hr)	5.08	5.09	5.30 (N=1)	4.83 (N=1)	5.00
CL/F (L/hr/kg)	0.096	0.107	0.084 (N=1)	0.076 (N=1)	0.100
V_z/F (L/kg)	0.700	0.781	0.645 (N=1)	0.532 (N=1)	0.718

Additional Information: Animals for the 0.5 and 5 mg/kg RM-493 in mPEG-DSPE groups received a 7-day washout period before being dosed with 10 mg/kg RM-493 in mPEG-DSPE and 5 mg/kg RM-493 in mPEG-DSPE diluted 1:1 in 5% mannitol, respectively.

Abbreviations: AUC = area under the plasma concentration-time curve; AUC_{∞} = AUC from time 0 to infinity; AUC_{∞}/Dose = dose-normalized AUC from time 0 to infinity; AUC_{Extrap} = the percentage of AUC_{∞} based on extrapolation; AUC_{last} = AUC from time 0 to the last measurable timepoint; AUC_{last}/Dose = dose-normalized AUC from time 0 to the last measurable timepoint; C_{last} = last measurable plasma concentration; CL/F = apparent plasma clearance; C_{max} = maximum plasma concentration; C_{max}/Dose= dose-normalized C_{max}; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; PK = pharmacokinetic; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; t_{last} = time of last measurable plasma concentration; t_{max} = time to C_{max}; V_z/F = apparent volume of distribution; λ_z = apparent elimination rate.

2.6.5.6.2 カニクイザルを用いた保存料添加及び非添加製剤のクロスオーバー薬物動態試験（RM-493-PK-007）

Monkey PK, Non-GLP	**Test Article:** Setmelanotide	
	Location in eCTD: 4.2.2.2-4	
	Study No.: RM-493-PK-007	
Species/Strain:	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**
Gender (M/F)/Number of animals:	6M	6M
Feeding condition:	Fed	Fed
Vehicle/Formulation:	RM-493 in clinical formulation + Preservative	RM-493 in clinical formulation + no Preservative
Method of Administration:	SC Injection	SC Injection
Dose (mg/kg):	5	5
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma
Analyte:	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS
Mean (± SD) PK parameters[a]:	**Phase 1**	**Phase 2**
t_{max} (hr)	9.33 ± 2.07	8.33 ± 1.97
C_{max} (ng/mL)	3,350 ± 727	2,990 ± 1,050
AUC_{last} (ng*hr/mL)	51,400 ± 6,540	49,100 ± 9,140
AUC_{∞} (ng*hr/mL)	51,400 ± 6,540	49,200 ± 9,130
AUC_{Extrap} (%)	0.146 ± 0.0368	0.274 ± 0.178
$t_{1/2}$ (hr)	4.59 ± 0.189	4.97 ± 0.460
CL/F (L/hr/kg)	0.0984 ± 0.011	0.104 ± 0.0168
V_z/F (L/kg)	0.652 ± 0.0826	0.748 ± 0.152

Additional Information: Phases 1 and 2 were separated by a 7-day washout period.
a) Data reported as Mean ± Standard Deviation (SD).

Abbreviations: AUC = area under the plasma concentration-time curve; AUC_{∞} = AUC from time 0 to infinity; AUC_{Extrap} = the percentage of AUC_{∞} based on extrapolation; AUC_{last} = AUC from time 0 to the last measurable timepoint; CL/F = apparent plasma clearance; C_{max} = maximum plasma concentration; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; PK = pharmacokinetic; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; t_{max} = time to C_{max}; V_z/F = apparent volume of distribution.

2.6.5.7 薬物動態：サルにおける反復投与時の吸収試験

2.6.5.7.1 カニクイザルを用いた 28 日間皮下投与用量設定試験（RM-493-TOX-027）

Monkey TK, Non-GLP	**Test Article:** Setmelanotide			
	Location in eCTD: 4.2.3.2-4			
	Study No.: RM-493-TOX-027			
Species/Strain:	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**
Gender (M/F)/Number of animals:	3M/3F	3M/3F	3M/3F	3M/3F
Feeding condition:	Fed	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in Saline	RM-493 in mPEG-DSPE	RM-493 in mPEG-DSPE	RM-493 in mPEG-DSPE
Method of Administration:	SC Injection	SC Injection	SC Injection	SC Injection
Dose (mg/kg/day):	2	0.2	0.75	2
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma	Plasma
Analyte:	RM-493	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Mean PK parameters (Day 1):				
t_{max} (hr) (Median)	0.500	4.00	6.00	8.00
C_{max} (ng/mL)	3,800	155	461	915
C_{max}/Dose ([ng/mL]/[mg/kg])	1,900	776	614	457
$AUC_{0-\tau}$ (ng*hr/mL)	14,500	1,330	5,400	13,600
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	7,250	6,670	7,210	6,810
AUC_{last} (ng*hr/mL)	14,500	1,190	5,400	13,600
AUC_{last}/Dose ([ng*hr/mL]/[mg/kg])	7,250	5,940	7,210	6,810
$t_{1/2}$ (hr)	NC	NC	NC	NC

Monkey TK, Non-GLP	**Test Article:** Setmelanotide			
	Location in eCTD: 4.2.3.2-4			
	Study No.: RM-493-TOX-027			
Species/Strain:	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**
Vehicle/Formulation:	RM-493 in Saline	RM-493 in mPEG-DSPE	RM-493 in mPEG-DSPE	RM-493 in mPEG-DSPE
Dose (mg/kg/day):	2	0.2	0.75	2
Mean PK parameters (Day 28):				
t_{max} (hr) (Median)	0.500	4.00	6.00	8.00
C_{max} (ng/mL)	3,940	152	437	1,060
C_{max}/Dose ([ng/mL]/[mg/kg])	1,970	760	583	528
$AUC_{0-\tau}$ (ng*hr/mL)	16,700	1,390	5,320	14,800
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	8,350	6,970	7,090	7,420
AUC_{last} (ng*hr/mL)	17,200	1,320	5,320	15,900
AUC_{last}/Dose ([ng*hr/mL]/[mg/kg])	8,610	6,590	7,090	7,950
$t_{1/2}$ (hr)	6.44	3.93	3.44	5.48
CL_{ss}/F (L/hr/kg)	0.122	0.144	0.143	0.135
V_z/F (L/kg)	1.12	0.764	0.759	1.07
R_{ac}[a]	1.15	1.05	0.986	1.10
Additional Information: a) R_{ac} = [$AUC_{0-\tau}$ (Day 28)] / [$AUC_{0-\tau}$ (Day 1)].				

Abbreviations: AUC = area under the plasma concentration-time curve; $AUC_{0-\tau}$ = AUC during a time interval; $AUC_{0-\tau}$/Dose = dose-normalized AUC during a time interval; AUC_{last} = AUC from time 0 to the last measurable timepoint; AUC_{last}/Dose = dose-normalized AUC from time 0 to the last measurable timepoint; CL_{ss}/F = apparent plasma clearance at steady state; C_{max} = maximum plasma concentration; C_{max}/Dose = dose-normalized C_{max}; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; NC = not calculated; PK = pharmacokinetic; R_{ac} = systemic accumulation ratio; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; TK = toxicokinetic; t_{max} = time to C_{max}; V_z/F = apparent volume of distribution.

2.6.5.7.2 カニクイザルを用いた 39 週間皮下投与毒性試験及び 4 週間回復性試験（RM-493-TOX-024）

Monkey TK, GLP	**Test Article:** Setmelanotide			
	Location in eCTD: 4.2.3.2-5			
	Study No.: RM-493-TOX-024			
Species/Strain:	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**
Gender (M/F)/Number of animals:	6M/6F	4M/4F	4M/4F	6M/6F
Feeding condition:	Fed	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in Saline	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol
Method of Administration:	SC Injection	SC Injection	SC Injection	SC Injection
Dose (mg/kg/day):	3	0.5/0.25[a]	1.5/0.5[a]	3/1[a]
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma	Plasma
Analyte:	RM-493	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Mean PK parameters (Day 1):				
t_{max} (hr) (Median)	0.500	4.00	10.0	9.00
C_{max} (ng/mL)	5,530	318	640	1,530
C_{max}/Dose ([ng/mL]/[mg/kg])	1,840	636	427	510
$AUC_{0-\tau}$ (ng*hr/mL)	22,300	3,300	9,090	20,100
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	7,450	6,600	6,060	6,710
AUC_{last} (ng*hr/mL)	22,300	3,170	9,090	20,100
AUC_{last}/Dose ([ng*hr/mL]/[mg/kg])	7,450	6,340	6,060	6,710
$t_{1/2}$ (hr)	NR	NR	NR	NR

Monkey TK, GLP	**Test Article:** Setmelanotide			
	Location in eCTD: 4.2.3.2-5			
	Study No.: RM-493-TOX-024			
Species/Strain:	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**
Vehicle/Formulation:	RM-493 in Saline	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol
Dose (mg/kg/day):	3	0.5/0.25[a]	1.5/0.5[a]	3/1[a]
Mean PK parameters (Week 13):				
t_{max} (hr) (Median)	0.500	2.00	3.00	3.00
C_{max} (ng/mL)	4,750	242	331	792
C_{max}/Dose ([ng/mL]/[mg/kg])	1,580	969	661	792
$AUC_{0-\tau}$ (ng*hr/mL)	18,800	2,540	3,180	7,590
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	6,270	10,100	6,360	7,590
AUC_{last} (ng*hr/mL)	18,800	2,480	3,100	7,590
AUC_{last}/Dose ([ng*hr/mL]/[mg/kg])	6,270	9,930	6,210	7,590
$t_{1/2}$ (hr)	3.87	4.56	3.37	4.97
CL_{ss}/F (L/hr/kg)	0.205	0.142	0.173	0.149
V_z/F (L/kg)	1.18	0.675	0.749	0.954
R_{ac}[b]	0.836	1.54	1.06	1.14
Mean PK parameters (Week 26):				
t_{max} (hr) (Median)	0.500	2.00	2.00	4.00
C_{max} (ng/mL)	4,750	368	372	1,100
C_{max}/Dose ([ng/mL]/[mg/kg])	1,580	1,470	744	1,100
$AUC_{0-\tau}$ (ng*hr/mL)	18,900	4,880	3,170	14,500
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	6,290	19,500	6,340	14,500
AUC_{last} (ng*hr/mL)	18,900	4,820	3,100	14,500
AUC_{last}/Dose ([ng*hr/mL]/[mg/kg])	6,290	19,300	6,200	14,500
$t_{1/2}$ (hr)	3.89	8.16	4.01	7.58
CL_{ss}/F (L/hr/kg)	0.254	0.116	0.163	0.121
V_z/F (L/kg)	1.62	0.640	0.894	0.875
R_{ac}[b]	0.883	3.01	1.06	2.19

Monkey TK, GLP	**Test Article**: Setmelanotide			
	Location in eCTD: 4.2.3.2-5			
	Study No.: RM-493-TOX-024			
Species/Strain:	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**	**Monkey/ Cynomolgus**
Vehicle/Formulation:	RM-493 in Saline	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol
Dose (mg/kg/day):	3	0.5/0.25[a]	1.5/0.5[a]	3/1[a]
Mean PK parameters (Week 39):				
t_{max} (hr) (Median)	0.500	2.00	3.00	4.00
C_{max} (ng/mL)	5,630	393	434	1,200
C_{max}/Dose ([ng/mL]/[mg/kg])	1,880	1,570	867	1,200
$AUC_{0-\tau}$ (ng*hr/mL)	22,100	4,860	3,510	15,200
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	7,370	19,400	7,020	15,200
AUC_{last} (ng*hr/mL)	22,100	4,800	3,490	15,200
AUC_{last}/Dose ([ng*hr/mL]/[mg/kg])	7,370	19,200	6,980	15,200
$t_{1/2}$ (hr)	4.05	9.03	5.30	11.2
CL_{ss}/F (L/hr/kg)	0.139	0.119	0.153	0.118
V_z/F (L/kg)	0.811	0.738	1.09	1.03
R_{ac}[b]	1.02	3.05	1.16	2.32

Additional Information:

a) Due to conditions at the dosing sites, the concentration of the formulation was reduced starting on Day 53/54 (during Week 8), and in consequence, the dose volume was increased and dose level decreased.

b) R_{ac} = Dose-normalized $AUC_{0-\tau}$ for Weeks 13, 26, and 39 divided by $AUC_{0-\tau}$ for Day 1.

Abbreviations: AUC = area under the plasma concentration-time curve; $AUC_{0-\tau}$ = AUC during a time interval; $AUC_{0-\tau}$/Dose = dose-normalized AUC during a time interval; AUC_{last} = AUC from time 0 to the last measurable timepoint; AUC_{last}/Dose = dose-normalized AUC from time 0 to last measurable timepoint; CL_{ss}/F = apparent plasma clearance at steady state; C_{max} = maximum plasma concentration; C_{max}/Dose = dose-normalized C_{max}; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; NR = not reported; PK = pharmacokinetic; R_{ac} = systemic accumulation ratio; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; TK = toxicokinetic; t_{max} = time to C_{max}; V_z/F = apparent volume of distribution.

2.6.5.8 薬物動態：がん原性モデルマウスにおける反復投与時の吸収試験

2.6.5.8.1 野生型 rasH2-Wt マウスの 28 日間皮下投与用量設定試験（RM-493-TOX-041）

Mouse TK, GLP	**Test Article:** Setmelanotide		
	Location in eCTD: 4.2.3.4.2-2		
	Study No.: RM-493-TOX-041		
Species/Strain:	**Mouse/ Wild Type rasH2[a]**	**Mouse/ Wild Type rasH2[a]**	**Mouse/ Wild Type rasH2[a]**
Gender (M/F)/Number of animals:	48M/48F	48M/48F	48M/48F
Feeding condition:	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol
Method of Administration:	SC Injection	SC Injection	SC Injection
Dose (mg/kg/day):	10 (Dose volume 10 mL/kg)	10 (Dose volume 5 mL/kg)	20 (Dose volume 5 mL/kg)
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma
Analyte:	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters (Day 1):			
t_{max} (hr)	1.00	1.00	2.00
C_{max} (ng/mL)	2,630	2,420	3,510
C_{max}/Dose ([ng/mL]/[mg/kg])	263	242	175
$AUC_{0-\tau}$ (ng*hr/mL)	8,720	8,260	18,200
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	872	826	911
AUC_{last} (ng*hr/mL)	8,660	8,120	17,100
AUC_{last}/Dose ([ng*hr/mL]/[mg/kg])	866	812	855
$t_{1/2}$ (hr)	1.03	1.19	1.70

Mouse TK, GLP	**Test Article:** Setmelanotide		
	Location in eCTD: 4.2.3.4.2-2		
	Study No.: RM-493-TOX-041		
Species/Strain:	**Mouse/ Wild Type rasH2[a]**	**Mouse/ Wild Type rasH2[a]**	**Mouse/ Wild Type rasH2[a]**
Vehicle/Formulation:	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol	RM-493 in mPEG-DSPE Diluted 1:10 with 5% Mannitol
Dose (mg/kg/day):	10 (Dose volume 10 mL/kg)	10 (Dose volume 5 mL/kg)	20 (Dose volume 5 mL/kg)
PK parameters (Day 28):			
t_{max} (hr)	1.00	1.00	2.00
C_{max} (ng/mL)	2,980	3,070	3,880
C_{max}/Dose ([ng/mL]/[mg/kg])	298	307	194
$AUC_{0-\tau}$ (ng*hr/mL)	10,400	9,600	20,000
$AUC_{0-\tau}$/Dose ([ng*hr/mL]/[mg/kg])	1,040	960	998
AUC_{last} (ng*hr/mL)	10,300	9,510	17,900
AUC_{last}/Dose ([ng*hr/mL]/[mg/kg])	1,030	951	893
$t_{1/2}$ (hr)	1.14	1.00	2.21
CL_{ss}/F (L/hr/kg)	0.961	1.04	1.00
V_z/F (L/kg)	1.57	1.50	3.20
R_{ac}[b]	1.19	1.16	1.10

Additional Information:
a) CByB6F1-Tg(HRAS)2Jic mice.
b) R_{ac} = [$AUC_{0-\tau}$ (Day 28)]/ [$AUC_{0-\tau}$ (Day 1)].

Abbreviations: AUC = area under the plasma concentration-time curve; $AUC_{0-\tau}$ = AUC during a time interval; $AUC_{0-\tau}$/Dose = dose-normalized AUC during a time interval; AUC_{last} = AUC from time 0 to the last measurable timepoint; AUC_{last}/Dose = dose-normalized AUC from time 0 to the last measurable timepoint; CL_{ss}/F = apparent plasma clearance at steady state; C_{max} = maximum plasma concentration; C_{max}/Dose = dose-normalized C_{max}; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; PK = pharmacokinetic; R_{ac} = systemic accumulation ratio; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; TK = toxicokinetic; t_{max} = time to C_{max}; V_z/F = apparent volume of distribution.

2.6.5.8.2 rasH2 トランスジェニックマウスの 6 ヵ月間皮下投与がん原性試験（1）（RM-493-TOX-042）

Mouse TK, GLP	**Test Article:** Setmelanotide		
	Location in eCTD: 4.2.3.4.2-3		
	Study No.: RM-493-TOX-042		
Species/Strain:	**Mouse/ Wild Type rasH2[a]**	**Mouse/ Wild Type rasH2[a]**	**Mouse/ Wild Type rasH2[a]**
Gender (M/F)/Number of animals:	12M/12F	12M/12F	12M/12F
Feeding condition:	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in mPEG-DSPE (10 mg/mL)	RM-493 in mPEG-DSPE (10 mg/mL)	RM-493 in mPEG-DSPE (10 mg/mL)
Method of Administration:	SC Injection	SC Injection	SC Injection
Dose (mg/kg/day):	1	3	10
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma
Analyte:	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters (Day 1, 1 hour post-dose, Mean [SD]):			
Concentration (ng/mL) Males	548 (95.1)	1,270 (203)	2,190 (656)
Concentration (ng/mL) Females	407 (55.7)	1,290 (463)	2,210 (457)
PK parameters (Day 182, 1 hour post-dose, Mean [SD]):			
Concentration (ng/mL) Males	480 (38.5)	1,430 (7.07)	2,140 (219)
Concentration (ng/mL) Females	375 (94.3)	1,080 (109)	1,660 (106)

Additional Information: Concentrations of RM-493 from the control and placebo groups receiving 0 mg/kg RM-493 were BLQ. ADA: Five (5) of the Day 183 samples tested screen-reactive and were analyzed in the confirmatory format. Three (3) samples were confirmed to be negative in the confirmatory assay. Two (2) of the screen-reactive samples belonging to subject 2040 and subject 3545 confirmed positive for antibodies to RM-493. The % inhibition was 14.2% and 20.6%, for these animals, respectively. Of these two samples, one was drawn from a drug-naïve vehicle control animal (subject 2040). The confirmatory cutpoint for this assay was 11.1%, and consequently, the sample from subject 2040 should be regarded as a false positive, as the animal received vehicle only. The result for animal 3545, a female dosed with 1 mg/kg RM-493, may also reflect a false positive. As separate animals were used for TK and ADA, it is not possible to determine if there is a correlation between the presence of ADA and any impact on TK; however, the Day 182 TK values were only slightly lower than the Day 1 samples for the 1 mg/kg dose group, with values of 428 and 478 ng/mL, respectively.

a) CByB6F1-Tg(HRAS)2Jic mice.

Abbreviations: ADA = anti-drug antibody; BLQ = below the limit of quantitation; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; PK = pharmacokinetic; RM-493 = setmelanotide; SC = subcutaneous; SD = standard deviation; TK = toxicokinetic.

2.6.5.8.3 rasH2 トランスジェニックマウスの 6 ヵ月間皮下投与がん原性試験（2）（RM-493-TOX-044）

Mouse TK, GLP	**Test Article:** Setmelanotide		
	Location in eCTD: 4.2.3.4.2-4		
	Study No.: RM-493-TOX-044		
Species/Strain:	**Mouse/ Wild Type rasH2[a]**	**Mouse/ Wild Type rasH2[a]**	**Mouse/ Wild Type rasH2[a]**
Gender (M/F)/Number of animals:	6M/6F	6M/6F	6M/6F
Feeding condition:	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in mPEG-DSPE (10 mg/mL)	RM-493 in mPEG-DSPE (10 mg/mL)	RM-493 in mPEG-DSPE (10 mg/mL)
Method of Administration:	SC Injection	SC Injection	SC Injection
Dose (mg/kg/day):	1	3	10
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma
Analyte:	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters (Day 182, 1 hour post-dose, Mean [SD]):			
Mean Concentration (ng/mL)	462 (95.6)	1,130 (239)	1,950 (735)
PK parameters (Day 182, 24 hour post-dose, Mean [SD]):			
Mean Concentration (ng/mL)	BLQ	BLQ	BLQ
Additional Information: Plasma concentrations of RM-493 from the control and placebo groups receiving 0 mg/kg RM-493 were BLQ at 1 and 24 hours post-dose. Samples from Day 183 were received and tested for each of 44 animals for anti-drug antibody to RM-493. Two (2) animals per sex were included for control groups and 6 animals per sex per group were included for groups treated with RM-493. Three (3) samples tested screen-reactive and were analyzed in the confirmatory format. All 3 samples confirmed negative. a) CByB6F1-Tg(HRAS)2Jic mice.			

Abbreviations: BLQ = below the limit of quantitation; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; RM-493 = setmelanotide; SC = subcutaneous; SD = standard deviation.

2.6.5.8.4 rasH2 トランスジェニックマウスの 6 ヵ月間皮下投与がん原性試験（3）（RM-493-TOX-045）

Mouse TK, GLP	**Test Article:** Setmelanotide		
	Location in eCTD: 4.2.3.4.2-5		
	Study No.: RM-493-TOX-045		
Species/Strain:	**Mouse/ Wild Type rasH2[a]**	**Mouse/ Wild Type rasH2[a]**	**Mouse/ Wild Type rasH2[a]**
Gender (M/F)/Number of animals:	6M/6F	6M/6F	6M/6F
Feeding condition:	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in mPEG-DSPE (10 mg/mL)	RM-493 in mPEG-DSPE (10 mg/mL)	RM-493 in mPEG-DSPE (10 mg/mL)
Method of Administration:	SC Injection	SC Injection	SC Injection
Dose (mg/kg/day):	1	3	10
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma
Analyte:	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters (Day 182, 1 hour post-dose, Mean [SD]):			
Mean Concentration (ng/mL) Males	458 (21.1)	1,160 (228)	2,150 (645)
Mean Concentration (ng/mL) Females	381 (98.1)	1,190 (96.4)	3,010 (609)
PK parameters (Day 182, 24 hour post-dose, Mean [SD]):			
Mean Concentration (ng/mL) Males	BLQ	BLQ	BLQ
Mean Concentration (ng/mL) Females	BLQ	BLQ	BLQ
Additional Information: All Day 182, 1-hour post-dose and 24-hour post-dose, TK samples from Groups 1 and 2 (Saline control and Placebo Groups) were BLQ (<5.00 ng/mL). ADA samples were not analyzed but the TK results are similar to prior studies, suggesting no ADA to RM-493. a) CByB6F1-Tg(HRAS)2Jic mice.			

Abbreviations: ADA = anti-drug antibody; BLQ = below the limit of quantitation; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; RM-493 = setmelanotide; SC = subcutaneous; SD = standard deviation; TK = toxicokinetic.

2.6.5.9 分布試験

2.6.5.9.1 In vitro ヒト血漿タンパク結合試験（T0106）

Plasma Protein Binding, Non-GLP		**Test Article:** Setmelanotide		
Study System: In Vitro – RM-493 was incubated for 30 minutes at 37°C with human plasma. Bound and free fractions were separated by ultrafiltration using Centrifree® and RM-493 levels were determined by LC-MS/MS.				
Target entity, Test System and Method: Bound and Free RM-493, Human Plasma, LC-MS/MS				
Species	**Concentration Tested (µmol/L)**	**% Bound**	**Study No.**	**Location in eCTD**
Human	1	45.5 ± 1.3	T0106	4.2.2.3-1
Human	10	46.9 ± 1.2	T0106	
Human	100	53.4 ± 0.9	T0106	
Additional Information: RM-493 appeared to bind to human plasma proteins by 46%, 47% and 53% at 1, 10 and 100 µmol/L, respectively.				

Abbreviations: eCTD = electronic Common Technical Document; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; RM-493 = setmelanotide.

2.6.5.9.2 In vitro ヒト、ラット、マウス及びサル血漿タンパク結合試験（[redacted]RHYTP4R1）

Plasma Protein Binding, Non-GLP			**Test Article:** Setmelanotide	
Study System: In Vitro – The objective of this study was to determine the percent bound of RM-493 in human, rat, mouse, and monkey plasma using equilibrium dialysis. A Rapid Equilibrium Dialysis device was used for all experiments. Stock solutions of the test article and control compound were first prepared in DMSO. Aliquots of the DMSO solutions were dosed into 1.0 mL of plasma at a dosing concentration of 5 µmol/L for the test article and 10 µmol/L for the co-dosed control compound, warfarin. Plasma (300 µL), containing test article and control compound, was loaded into two wells of the 96-well dialysis plate. Blank phosphate-buffered saline (500 µL) was added to each corresponding receiver chamber. The device was then placed into an enclosed heated rocker that was pre-warmed to 37°C, and allowed to incubate for 4 hr. After incubation, both sides were sampled. RM-493 levels were determined by LC-MS/MS.				
Target entity, Test System and Method: Bound and Free RM-493, Plasma, LC-MS/MS				
Species	**Concentration Tested (µmol/L)**	**% Bound**	**Study No.**	**Location in eCTD**
Human	5	79.1	[redacted]RHYTP4R1	4.2.2.3-2
Rat	5	82.1	[redacted]RHYTP4R1	
Mouse	5	82.4	[redacted]RHYTP4R1	
Monkey	5	78.3	[redacted]RHYTP4R1	
Additional Information: Warfarin, the control compound, met all of the binding acceptance criteria in this experiment. In addition, RM-493 binding to rat plasma proteins was similar to that obtained in Study [redacted]RHYTP5 (2.6.5.9.3), at 80.6% binding.				

Abbreviations: eCTD = electronic Common Technical Document; DMSO = dimethyl sulfoxide; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; RM-493 = setmelanotide.

2.6.5.9.3 In vitro ラット及びウサギ血漿タンパク結合試験（[redacted]RHYTP5）

Plasma Protein Binding, Non-GLP			**Test Article:** Setmelanotide	
Study System: In vitro – The objective of this study was to determine the percent bound of RM-493 in rat and rabbit plasma using equilibrium dialysis. A Rapid Equilibrium Dialysis device was used for all experiments. Stock solutions of the test article and control compound were first prepared in DMSO. Aliquots of the DMSO solutions were dosed into 1.0 mL of plasma at a dosing concentration of 5 µmol/L for the test article and 10 µmol/L for the co-dosed control compound, warfarin. Plasma (300 µL), containing test article and control compound, was loaded into two wells of the 96-well dialysis plate. Blank phosphate-buffered saline (500 µL) was added to each corresponding receiver chamber. The device was then placed into an enclosed heated rocker that was pre-warmed to 37°C, and allowed to incubate for 4 hr. After incubation, both sides were sampled. RM-493 levels were determined by LC-MS/MS.				
Target entity, Test System and Method: LC-MS/MS				
Species	**Concentration Tested (µmol/L)**	**% Bound**	**Study No.**	**Location in eCTD**
Rat	5	80.6	[redacted]RHYTP5	4.2.2.3-3
Rabbit	5	76.3	[redacted]RHYTP5	
Additional Information: Warfarin, the control compound, met all of the binding acceptance criteria in this experiment. In addition, RM-493 binding to rat plasma proteins was similar to that obtained in [redacted]RHYTP4R1 (2.6.5.9.2), at 82.1% binding.				

Abbreviations: eCTD = electronic Common Technical Document; DMSO = dimethyl sulfoxide; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; RM-493 = setmelanotide.

2.6.5.9.4 雄ラットにおける in vivo 組織分布試験（RM-493-PK-008）

QWBA-males, Non-GLP	**Test Article:** [^{14}C]-Setmelanotide
	Location in eCTD: 4.2.2.2-1
	Study No.: RM-493-PK-008
Species/Strain:	Rat / Sprague-Dawley
Gender (M/F)/Number of animals:	7M
Feeding condition:	Fed
Vehicle/Formulation:	[^{14}C]-RM-493 in Saline
Method of Administration:	SC Injection
Dose:	2 mg/kg (100 µCi/kg)
Radionuclide:	^{14}C
Specific Activity:	339 mCi/mmol
Sampling Time:	0.083, 0.5, 1, 4, 8, 24, 168 hr
Method of Analysis:	Autoradiography

Males	**PK Parameter**							
Tissues/organs	**C_{max} (ng equiv./g)**	**t_{max} (hr)**	**$t_{1/2}$ (hr)**	**AUC_{last} (ng equiv.*hr/g)**	**AUC_{∞} (ng equiv.*hr/g)**	**AUC_{Extrap} (%)**	**R^2**	**Tissue/ Plasma[a]**
Plasma	1,770	1	79.8	16,900	19,800	15.1	0.974	1.00
Whole Blood	1,020	1	176	15,000	31,800	52.9	0.180	0.89
Adrenal Gland	601	1	114	41,000	61,400	33.2	0.951	2.43
Bone marrow (Femur)	405	24	NC	44,100	NC	NC	NA	2.61
Bone (Femur)	807	0.5	178	19,500	38,300	49.2	0.971	1.15
Brain	65.1	168	NC	9,600	NC	NC	NA	0.57
Eyes (Lens)	93.8	0.083	NC	37.8	NC	NC	NA	0.00
Fat (Yellow)	232	1	NC	994	NC	NC	NA	0.06
Harderian Gland	441	1	104	27,300	41,100	33.6	0.947	1.62
Heart	536	1	323	19,000	59,600	68.2	0.991	1.12
Kidney	7,530	8	NC	448,000	NC	NC	NA	26.5
Kidney (Cortex)	10,600	8	NC	621,000	NC	NC	NA	36.7
Kidney (Medulla)	4,450	0.5	49.8	241,000	264,000	8.68	0.994	14.3
Large intestine (Wall)	512	1	82.5	26,200	34,000	23.0	0.992	1.55
Liver	1,610	4	61.0	56,300	66,900	15.9	0.820	3.33
Lungs	1,190	1	119	25,700	39,500	34.9	0.939	1.52

QWBA-males, Non-GLP	Test Article: [^{14}C]-Setmelanotide Location in eCTD: 4.2.2.2-1 Study No.: RM-493-PK-008							
Males	**PK Parameter**							
Tissues/organs	**C_{max} (ng equiv./g)**	**t_{max} (hr)**	**$t_{1/2}$ (hr)**	**AUC_{last} (ng equiv.*hr/g)**	**AUC_{∞} (ng equiv.*hr/g)**	**AUC_{Extrap} (%)**	**R^2**	**Tissue/ Plasma[a]**
Muscle (Femoral)	336	0.5	235	14,400	42,800	66.5	0.109	0.85
Pancreas	504	8	NC	36,000	NC	NC	NA	2.13
Pituitary gland	421	0.5	120	38,300	58,600	34.6	0.911	2.27
Prostate	827	0.5	117	36,500	51,700	29.4	0.601	2.16
Skin (non-pigmented)	800	1	218	18,600	42,700	56.4	0.834	1.10
Small intestine (wall)	582	0.5	76.9	35,700	44,100	19.0	0.976	2.11
Spleen	699	4	69.4	41,200	51,100	19.4	0.915	2.44
Stomach (wall)	862	0.5	104	29,700	42,500	30.0	0.638	1.76
Testes	189	0.5	230	11,600	33,300	65.1	0.205	0.69
Thymus	235	0.5	237	20,000	48,700	59.0	0.583	1.18
Thyroid gland	790	0.5	246	67,900	171,000	60.3	0.912	4.02
Uveal Tract	600	1	NC	2,740	NC	NC	NA	0.16

Additional Information:
a) Tissue/plasma ratio based on AUC_{last}.

Abbreviations: AUC = area under the concentration-time curve; AUC_{∞} = AUC from time 0 to infinity; AUC_{Extrap} = the percentage of AUC_{∞} based on extrapolation; AUC_{last} = AUC from time 0 to the last measurable timepoint; C_{max} = maximum concentration; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; M = male; NA = not applicable; NC = not calculated due to insufficient data; PK = pharmacokinetic; QWBA = quantitative whole-body autoradiography; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; t_{max} = time to C_{max}.

2.6.5.9.5 雌ラットにおける in vivo 組織分布試験（RM-493-PK-008）

QWBA-females, Non-GLP	**Test Article:** [^{14}C]-Setmelanotide
	Location in eCTD: 4.2.2.2-1
	Study No.: RM-493-PK-008
Species/Strain:	Rat/Sprague-Dawley
Gender (M/F)/Number of animals:	7F
Feeding condition:	Fed
Vehicle/Formulation:	[^{14}C]-RM-493 in Saline
Method of Administration:	Single SC Injection
Dose:	2 mg/kg (100 µCi/kg)
Radionuclide:	^{14}C
Specific Activity:	339 mCi/mmol
Sampling Time:	0.083, 0.5, 1, 4, 8, 24, 168 hr
Method of Analysis:	Autoradiography

Females	**PK Parameter**							
Tissues/organs	**C_{max} (ng equiv./g)**	**t_{max} (hr)**	**$t_{1/2}$ (hr)**	**AUC_{last} (ng equiv.*hr/g)**	**AUC_{∞} (ng equiv.*hr/g)**	**AUC_{Extrap} (%)**	**R^2**	**Tissue/ Plasma[a]**
Plasma	1,440	0.5	77.2	12,600	15,000	16.4	0.971	1.00
Whole Blood	850	0.5	362	10,200	32,700	68.8	0.621	0.81
Adrenal Gland	430	0.5	153	23,700	45,100	47.4	0.796	1.88
Bone marrow (Femur)	340	0.5	93.8	22,500	32,900	31.6	0.860	1.79
Bone (Femur)	501	1	NC	1,750	NC	NC	NA	1.14
Brain	63.1	0.5	NC	13.2	NC	NC	NA	0.00
Eyes (Lens)	NC	NC	NC	NC	NC	NC	NA	NC
Fat (Yellow)	191	1	NC	898	NC	NC	NA	0.07
Harderian Gland	362	1	176	15,200	32,900	53.8	0.763	1.21
Heart	557	0.5	129	13,700	28,100	51.2	0.248	1.09
Kidney	8,090	8	NC	377,000	NC	NC	NA	29.9
Kidney (Cortex)	11,700	8	NC	454,000	NC	NC	NA	36.0
Kidney (Medulla)	4,050	8	NC	293,000	NC	NC	NA	23.3
Large intestine (Wall)	697	0.5	90.6	22,600	32,000	29.2	0.873	1.79
Liver	786	8	NC	34,700	NC	NC	NA	2.75
Lungs	1,090	1	90.7	17,300	25,900	33.2	0.524	1.37

QWBA-females, Non-GLP	Test Article: [^{14}C]-Setmelanotide							
	Location in eCTD: 4.2.2.2-1							
	Study No.: RM-493-PK-008							
Females	**PK Parameter**							
Tissues/organs	**C_{max} (ng equiv./g)**	**t_{max} (hr)**	**$t_{1/2}$ (hr)**	**AUC_{last} (ng equiv.*hr/g)**	**AUC_{∞} (ng equiv.*hr/g)**	**AUC_{Extrap} (%)**	**R^2**	**Tissue/ Plasma[a]**
Muscle (Femoral)	220	1	NC	6,260	NC	NC	NA	0.50
Ovaries	937	0.5	92.1	19,800	29,100	31.9	0.733	1.57
Pancreas	469	8	NC	22,300	NC	NC	NA	1.77
Pituitary gland	545	0.5	138	21,500	38,300	43.9	0.890	1.71
Skin (non-pigmented)	621	1	97.0	7,910	16,200	51.2	0.879	0.63
Small intestine (wall)	617	1	30.8	5,970	13,400	55.3	1.00	0.47
Spleen	494	8	NC	23,100	NC	NC	NA	1.83
Stomach (wall)	675	0.5	169	16,100	31,100	48.1	0.971	1.28
Thymus	272	1	385	14,200	55,500	74.5	0.683	1.13
Thyroid gland	676	1	184	27,000	61,100	55.8	0.571	2.14
Uterus	1,200	0.5	76.5	19,600	29,200	33.0	0.426	1.56
Uveal Tract	109	1	NC	343	NC	NC	NA	0.03

Additional Information:
a) Tissue/plasma ratio based on AUC_{last}.

Abbreviations: AUC = area under the concentration-time curve; AUC_{∞} = AUC from time 0 to infinity; AUC_{Extrap} = the percentage of AUC_{∞} based on extrapolation; AUC_{last} = AUC from time 0 to the last measurable timepoint; C_{max} = maximum concentration; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; M = male; NA = not applicable; NC = not calculated due to insufficient data; PK = pharmacokinetic; QWBA = quantitative whole-body autoradiography; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; t_{max} = time to C_{max}.

2.6.5.9.6 雄 Long-Evans ラットにおける in vivo 組織分布試験（RM-493-PK-008）

QWBA-males, Non-GLP	**Test Article:** [^{14}C]-Setmelanotide
	Location in eCTD: 4.2.2.2-1
	Study No.: RM-493-PK-008
Species/Strain:	Rat/Long-Evans
Gender (M/F)/Number of animals:	9M
Feeding condition:	Fed
Vehicle/Formulation:	[^{14}C]-RM-493 in Saline
Method of Administration:	SC Injection
Dose:	2 mg/kg (100 µCi/kg)
Radionuclide:	^{14}C
Specific Activity:	339 mCi/mmol
Sampling Time:	0.083, 0.5, 1, 4, 8, 24, 168, 336, and 504 hr
Method of Analysis:	Autoradiography

Males	**PK Parameter**							
Tissues/organs	**C_{max} (ng equiv./g)**	**t_{max} (hr)**	**$t_{1/2}$ (hr)**	**AUC_{last} (ng equiv.*hr/g)**	**AUC_{∞} (ng equiv.*hr/g)**	**AUC_{Extrap} (%)**	**R^2**	**Tissue/ Plasma[a]**
Plasma	2,150	1	121	22,500	23,800	5.15	0.942	1.00
Whole Blood	1,200	1	624	35,500	81,600	56.5	0.942	1.58
Eye (Lens)	103	504	NC	30,600	NC	NC	NA	1.36
Fat (Yellow)	160	1	NC	697	NC	NC	NA	0.03
Skin (Pigmented)	834	1	111	17,200	28,100	39.0	0.638	0.76
Uveal tract	1,430	1	21,250	38,700	235,000	83.5	0.998	1.72

Additional Information:
a) Tissue/plasma ratio based on AUC_{last}.

Abbreviations: AUC = area under the concentration-time curve; AUC_{∞} = AUC from time 0 to infinity; AUC_{Extrap} = the percentage of AUC_{∞} based on extrapolation; AUC_{last} = AUC from time 0 to the last measurable timepoint; C_{max} = maximum concentration; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; M = male; NA = not applicable; NC = not calculated due to insufficient data; PK = pharmacokinetic; QWBA = quantitative whole-body autoradiography; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; t_{max} = time to C_{max}.

2.6.5.9.7 [^{14}C]-mPEG-DSPE を単回及び反復皮下投与時の PK、マスバランス及び組織分布試験（RM-493-PK-013）

Rat PK, Non-GLP	**Test Article:** [^{14}C]-mPEG-DSPE
	Location in eCTD: 4.2.2.3-4
	Study No.: RM-493-PK-013
Species/Strain:	Rat /Sprague-Dawley
Gender (M/F)/Number of Animals:	PK: 12M/12F; Mass Balance: 3M/3F; QWBA: 1M/1F; TD: 3M/3F for Single Dose and 12M/12F for Multiple Dose
Feeding condition:	Fed
Vehicle/Formulation:	[^{14}C]-mPEG-DSPE in 5% mannitol in water
Method of Administration:	SC Injection
Dose:	20 mg/kg (100 µCi/kg for PK, Mass Balance and Single Dose Phase of TD; 10 µCi/kg/Day × 7 QD for QWBA and Multiple Dose Phase of TD)
Radionuclide:	^{14}C
Specific Activity:	9.52 µCi/mg
Sampling Time:	0.0, 0.5, 1, 2, 4, 8, 12, 24 hour, Day 2, Day 3, Day 4, Day 5, Day 6, Day 7, Day 10, Day 14
Method of Analysis:	LSC for PK analysis, TD and Mass Balance; QWBA for TD

Males	**PK Parameter**						
Tissues/organs	**C_{max} (µg equiv./g)**	**t_{max} (hr)**	**$t_{1/2}$ (hr)**	**AUC_{last} (µg equiv.*hr/g)**	**AUC_{∞} (µg equiv.*hr/g)**	**AUC_{Extrap} (%)**	**R^2**
Plasma (Males)	33.3	8	43.2	544	548	0.598	0.970
Plasma (Females)	30.1	8	30.1	508	509	0.197	1.00

QWBA, Non-GLP	Test Article: [^{14}C]-mPEG-DSPE									
	Location in eCTD: 4.2.2.3-4									
	Study No.: RM-493-PK-013									
Plasma and Tissue Concentrations (µg equiv./g)										
Tissue	Day 1, 24 hours		Day 7, 24 hours		Day 7, 2 Weeks		Day 7, 7 Weeks		Day 7, 12 Weeks	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Plasma	6.81	6.23	6.55	6.85	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Whole Blood	4.21	3.78	4.51	5.12	BLQ	0.18	BLQ	BLQ	BLQ	BLQ
Adrenal gland	14.5	10.1	38.6	40.3	NA	NA	NA	NA	BLQ	BLQ
Bone Marrow	8.90	5.71	9.80	9.58	NA	NA	NA	NA	BLQ	BLQ
Bone (Femur)	2.64	1.92	5.41	4.09	NA	NA	NA	NA	BLQ	BLQ
Brain	0.29	0.22	0.68	0.55	NA	NA	NA	NA	BLQ	BLQ
Choroid Plexus	6.31	4.83	BLQ	BLQ	BLQ	BLQ	BLQ	18.5	BLQ	BLQ
Eye	0.67	0.65	0.84	1.42	NA	NA	NA	NA	BLQ	BLQ
Fat	0.65	0.90	1.89	1.30	NA	NA	NA	NA	BLQ	BLQ
GI Tract	6.52	8.81	9.44	9.39	NA	NA	NA	NA	0.07	0.05
GI Tract Contents	15.3	24.1	18.1	22.7	NA	NA	NA	NA	BLQ	BLQ
Heart	2.33	1.99	3.28	3.36	NA	NA	NA	NA	BLQ	BLQ
Injection Site	72.4	117	49.0	53.1	NA	NA	NA	NA	BLQ	BLQ
Kidney	9.45	7.54	13.6	12.7	NA	NA	NA	NA	0.06	0.06
Liver	9.67	7.87	15.2	14.7	NA	NA	NA	NA	BLQ	0.06
Lung	7.49	5.33	9.46	9.74	NA	NA	NA	NA	0.33	BLQ
Mesenteric Lymph Node	1.50	1.83	3.21	5.05	NA	NA	NA	NA	BLQ	BLQ
Muscle (Femoral)	1.41	0.62	1.92	2.15	NA	NA	NA	NA	0.11	0.11
Ovary	NA	4.96	NA	8.77	NA	NA	NA	NA	NA	BLQ
Pancreas	3.17	2.56	4.36	5.56	NA	NA	NA	NA	BLQ	BLQ
Prostate	2.78	NA	5.32	NA	NA	NA	NA	NA	BLQ	NA
Skin	3.12	5.46	7.66	6.45	NA	NA	NA	NA	BLQ	BLQ
Spleen	12.5	9.07	25.6	22.6	NA	NA	NA	NA	BLQ	0.02
Testes w/Epididymis	2.30	NA	6.52	NA	NA	NA	NA	NA	0.23	NA
Thymus	6.80	5.89	12.1	9.94	NA	NA	NA	NA	0.09	BLQ
Thyroid gland	3.69	2.68	15.6	8.76	NA	NA	NA	NA	0.76	BLQ
Uterus	NA	4.51	NA	7.38	NA	NA	NA	NA	NA	BLQ
Carcass	3.19	4.04	7.36	8.93	0.71	0.80	0.20	0.18	0.03	0.10

Rat Excretion, Non-GLP		**Test Article**: [^{14}C]-mPEG-DSPE											
		Location in eCTD: 4.2.2.3-4											
		Study No.: RM-493-PK-013											
		% Dose Recovered in Urine, Feces, Cage Rinse, and Cage Wash											
		Males						**Females**					
	Collection Time	**Animal 47010**	**Animal 47023**	**Animal 47040**	**Mean**	**SD**	**Cumul-ative**	**Animal 47049**	**Animal 47057**	**Animal 47075**	**Mean**	**SD**	**Cumul-ative**
Urine	0 – 6 hr	7.85	7.96	5.47	7.09	1.41	7.09	5.67	0.77	1.72	2.72	2.60	2.72
	6 – 24 hr	44.1	46.8	44.3	45.1	1.49	52.2	46.5	49.2	50.8	48.8	2.14	51.6
	24 – 48 hr	10.7	10.4	12.2	11.1	0.98	63.3	9.50	10.1	10.2	9.94	0.39	61.5
	48 – 72 hr	2.97	2.83	2.99	2.93	0.09	66.2	2.63	2.65	2.67	2.65	0.02	64.1
	72 – 96 hr	1.62	1.38	1.50	1.50	0.12	67.7	1.31	1.28	1.36	1.32	0.04	65.5
	96 – 120 hr	0.86	0.84	0.96	0.89	0.06	68.6	0.94	0.83	0.93	0.90	0.06	66.4
	120 – 144 hr	0.63	0.56	0.57	0.58	0.04	69.2	0.56	0.52	0.65	0.58	0.06	66.9
	144 – 168 hr	0.44	0.38	0.39	0.40	0.03	69.6	0.40	0.31	0.46	0.39	0.08	67.3
	168 – 192 hr	0.32	0.26	0.30	0.29	0.03	69.9	0.25	0.27	0.33	0.28	0.04	67.6
	192 – 216 hr	0.22	0.17	0.19	0.20	0.03	70.1	0.18	0.17	0.23	0.19	0.03	67.8
	216 – 240 hr	0.16	0.13	0.15	0.15	0.02	70.2	0.16	0.14	0.18	0.16	0.02	68.0
	240 – 264 hr	0.12	0.09	0.10	0.10	0.02	70.3	0.11	0.09	0.12	0.10	0.01	68.1
	264 – 288 hr	0.09	0.07	0.08	0.08	0.01	70.4	0.08	0.06	0.10	0.08	0.02	68.1
	288 – 312 hr	0.08	0.06	0.06	0.07	0.01	70.5	0.06	0.05	0.08	0.06	0.02	68.2
	312 – 336 hr	0.06	0.04	0.05	0.05	0.01	70.5	0.05	0.05	0.07	0.06	0.01	68.3
	Sub-total	70.2	72.0	69.4	70.5	1.30		68.4	66.4	69.9	68.3	1.74	
Feces	0 – 24 hr	17.1	14.0	13.2	14.8	2.06	14.8	3.69	13.8	12.1	9.85	5.40	9.85
	24 – 48 hr	5.29	6.01	7.23	6.18	0.98	20.9	17.5	11.8	1.65	10.31	8.04	20.2
	48 – 72 hr	0.81	0.76	1.40	0.99	0.35	21.9	2.12	1.20	1.04	1.46	0.58	21.6
	72 – 96 hr	0.30	0.20	0.35	0.29	0.08	22.2	0.27	0.37	0.31	0.32	0.05	21.9
	96 – 120 hr	0.14	0.20	0.11	0.15	0.04	22.4	0.13	0.15	0.18	0.15	0.03	22.1
	120 – 144 hr	0.08	0.05	0.13	0.09	0.04	22.5	0.22	0.13	0.10	0.15	0.06	22.2
	144 – 168 hr	0.05	0.04	0.05	0.05	0.01	22.5	0.09	0.06	0.07	0.07	0.02	22.3
	168 – 192 hr	0.03	0.03	0.04	0.04	0.01	22.5	0.05	0.03	0.06	0.05	0.02	22.4
	192 – 216 hr	0.02	0.03	0.03	0.03	0.00	22.6	0.03	0.04	0.04	0.04	0.01	22.4
	216 – 240 hr	0.02	0.02	0.02	0.02	0.00	22.6	0.05	0.02	0.02	0.03	0.02	22.4
	240 – 264 hr	0.02	0.01	0.01	0.01	0.00	22.6	0.06	0.02	0.03	0.03	0.02	22.5
	264 – 288 hr	0.01	0.01	0.01	0.01	0.00	22.6	0.02	0.01	0.03	0.02	0.01	22.5

Rat Excretion, Non-GLP		**Test Article**: [^{14}C]-mPEG-DSPE											
		Location in eCTD: 4.2.2.3-4											
		Study No.: RM-493-PK-013											
		% Dose Recovered in Urine, Feces, Cage Rinse, and Cage Wash											
		Males						**Females**					
	Collection Time	**Animal 47010**	**Animal 47023**	**Animal 47040**	**Mean**	**SD**	**Cumul-ative**	**Animal 47049**	**Animal 47057**	**Animal 47075**	**Mean**	**SD**	**Cumul-ative**
	288 – 312 hr	0.01	0.01	0.01	0.01	0.00	22.6	0.01	0.01	0.02	0.02	0.01	22.5
	312 – 336 hr	0.01	0.01	0.01	0.01	0.00	22.6	0.01	0.02	0.01	0.01	0.00	22.5
	Sub-total	23.9	21.4	22.6	22.6	1.25		24.3	27.6	15.6	22.5	6.17	
Cage Rinse	0 – 6 hr	0.74	1.18	0.53	0.82	0.33	0.82	0.94	0.09	0.36	0.46	0.43	0.46
	6 – 24 hr	1.92	2.21	2.53	2.22	0.30	3.04	2.22	2.63	2.50	2.45	0.21	2.91
	24 – 48 hr	0.63	0.56	0.72	0.64	0.08	3.67	0.44	0.88	0.69	0.67	0.22	3.58
	48 – 72 hr	0.22	0.17	0.23	0.20	0.03	3.88	0.25	0.29	0.25	0.26	0.02	3.85
	72 – 96 hr	0.13	0.12	0.15	0.13	0.02	4.01	0.18	0.20	0.15	0.18	0.03	4.03
	96 – 120 hr	0.07	0.07	0.09	0.08	0.01	4.09	0.13	0.09	0.10	0.11	0.02	4.13
	120 – 144 hr	0.08	0.04	0.08	0.07	0.02	4.16	0.07	0.11	0.07	0.08	0.02	4.22
	144 – 168 hr	0.03	0.05	0.04	0.04	0.01	4.20	0.08	0.05	0.05	0.06	0.01	4.28
	168 – 192 hr	0.03	0.03	0.06	0.04	0.02	4.24	0.05	0.04	0.05	0.05	0.01	4.33
	192 – 216 hr	0.01	0.02	0.03	0.02	0.01	4.26	0.03	0.03	0.03	0.03	0.00	4.36
	216 – 240 hr	0.02	0.02	0.03	0.02	0.01	4.28	0.03	0.02	0.03	0.03	0.01	4.38
	240 – 264 hr	0.01	0.01	0.01	0.01	0.00	4.29	0.01	0.02	0.01	0.01	0.00	4.40
	264 – 288 hr	0.01	0.00	0.02	0.01	0.01	4.30	0.01	0.02	0.01	0.01	0.00	4.41
	288 – 312 hr	0.01	0.01	0.02	0.01	0.00	4.31	0.01	0.01	0.01	0.01	0.00	4.42
	312 – 336 hr	0.00	0.01	0.01	0.01	0.00	4.32	0.01	0.01	0.02	0.01	0.00	4.43
	Sub-total	3.92	4.49	4.54	4.32	0.34		4.47	4.50	4.33	4.43	0.09	
Cage Wash	336 hr	0.01	0.02	0.02	0.01	0.01		0.02	0.04	0.01	0.03	0.01	
Total		98.1	97.8	96.6	97.5	0.81		97.2	98.6	89.9	95.2	4.67	
Additional Information: None.													

Abbreviations: AUC = area under the plasma concentration-time curve; AUC_{∞} = AUC from time 0 to infinity; AUC_{Extrap} = the percentage of AUC_{∞} based on extrapolation; AUC_{last} = AUC from time 0 to the last measurable timepoint; BLQ = below the limit of quantitation; C_{max} = maximum plasma concentration; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LSC = liquid scintillation counting; M = male; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; NA = not applicable; PK = pharmacokinetic; QD = quaque die (once daily); QWBA = quantitative whole-body autoradiography; RM-493 = setmelanotide; SC = subcutaneous; SD = standard deviation; $t_{1/2}$ = half-life; TD = tissue distribution; t_{max} = time to C_{max}.

2.6.5.10 In vitro 代謝

2.6.5.10.1 ヒト肝細胞における代謝安定性試験（T0059）

Metabolic Stability, Non-GLP	**Test Article:** Setmelanotide
	Location in eCTD: 4.2.2.4-1
	Study No.: T0059

Study System: In vitro – The aim of this study was to evaluate the metabolic stability of RM-493 in the presence of cryopreserved human hepatocytes and to identify the potential metabolites and/or the transformation products.
Hepatocytes with test article(s) were incubated in a water bath at 37°C under agitation. Samples were collected after various incubation times.

Time:	0, 30, 60, 120 min for RM-493; 0. 15, 30, 60 min for testosterone
Concentration:	50 µmol/L for RM-493; 5 µmol/L for testosterone
Method of Analysis:	LC-UV

Half-life and Clearance of Testosterone and RM-493 in Human Cryopreserved Hepatocytes

Compound	**Half-life (min) [95% Confidence Interval Limits]**	**CL_{int} (µL/min/10^6 cells) [95% Confidence Interval Limits]**
Testosterone	14.1 [6.6 – 21.6]	87.8 [41.1 – 134.6]
IRC-022493-JM/1 (RM-493)	>120	NA

Additional Information: RM-493 was not metabolized by human cryopreserved hepatocytes under the conditions applied. The appearance of a peak was observed immediately after the compound was added to the cells with no time dependent evolution. RM-493 was not toxic within the 120 min-incubation with hepatocytes. The testosterone results were in agreement with reports from the literature.

Abbreviations: CL_{int} = intrinsic clearance; eCTD = electronic Common Technical Document; GLP = Good Laboratory Practice; LC-UV = liquid chromatography with ultraviolet detection; NA = not applicable; RM-493 = setmelanotide.

2.6.5.10.2 ヒト肝ミクロソームにおける代謝安定性試験（T0076）

Metabolic Stability, Non-GLP	**Test Article:** Setmelanotide
	Location in eCTD: 4.2.2.4-2
	Study No.: T0076

Study System: In vitro – This study was to evaluate the metabolism of RM-493 by human liver microsomes at 37°C and to determinate the half-life and intrinsic clearance (CL_{int}) of the parent compound. The microsomal suspensions in Tris buffer (testosterone) or PO_4 (RM-493) buffer were preincubated at 37°C for 5 min with the compound. The addition of NADPH started the reactions. During the incubation, at each predefined time, the suspensions were transferred to a microtiter plate containing 200 µL acetonitrile and maintained 30 min at 4°C in order to quench the reaction. Then, the precipitated proteins were eliminated by centrifugation (1,200 rpm) at 4°C for 20 min. Supernatants were then either directly injected on the HPLC system (50 µL) or kept frozen at -80°C pending analysis.

Time:	0, 10, 20, 40 min
Concentration:	10 µmol/L for RM-493; 200 µmol/L for Testosterone
Method of Analysis:	LC-UV

Half-life and Clearance of Testosterone and RM-493 in Human Liver Microsomes

Compound	**Half-life (min) [95% Confidence Interval Limits]**	**CL_{int} (µL/min/mg protein) [95% Confidence Interval Limits]**
Testosterone	28.4 [14.3 – 46.3]	22.5 [13.8 – 31.2]
IRC-022493-JM/1 (RM-493)	ND	ND

Additional Information: Under the experimental conditions, RM-493 is not transformed by human liver microsomes, whereas testosterone (positive control) transformation rate was within the expected confidence interval.

Abbreviations: CL_{int} = intrinsic clearance; eCTD = electronic Common Technical Document; GLP = Good Laboratory Practice; HPLC = high performance liquid chromatography; LC-UV = liquid chromatography with ultraviolet detection; NADPH = nicotinamide adenine dinucleotide phosphate; ND = not determined; RM-493 = setmelanotide.

2.6.5.10.3 ヒト皮膚線維芽細胞における代謝安定性試験（T0115）

Metabolic Stability, Non-GLP	**Test Article:** Setmelanotide
	Location in eCTD: 4.2.2.4-3
	Study No.: T0115

Study System: In vitro – The aim of this study was to evaluate the metabolic stability of RM-493 in the presence of Hs68 human skin fibroblasts and identify the potential metabolites and/or the transformation products.

Time:	0, 60, 180, 360, 1,440 min for RM-493; 0, 60, 180, 360 min for Substance P
Concentration:	50 µmol/L for RM-493; 50 µmol/L for Substance P
Method of Analysis:	LC-UV

Compound	**Half-life (min) [95% Confidence Interval Limits]**	**CL_{int} (µL/min/10^6 cells) [95% Confidence Interval Limits]**
Substance P	213 [115 – 310]	27.6 [13.1 – 42]
IRC-022493-JM/1 (RM-493)	21412 [0 – 76,920][a]	0 [0 – 1.7]

Additional Information: Under the experimental conditions, RM-493 was not metabolized by Hs68, a cell line of human skin fibroblasts. Substance P (positive control) was degraded in the presence of Hs68 human skin fibroblasts, with a 213 min half-life and 27.6 µL/min/10^6 cells intrinsic clearance. This result is in agreement with data from the literature reporting a degradation of substance P by fresh human muscle-derived fibroblasts. Under these experimental conditions, the half-life time is longer and might be a consequence of a weaker expression of cell-surface peptidases in Hs68 which is an immortalized cell line.

a) Extrapolated value.

Abbreviations: CL_{int} = intrinsic clearance; eCTD = electronic Common Technical Document; GLP = Good Laboratory Practice; LC-UV = liquid chromatography with ultraviolet detection; RM-493 = setmelanotide.

2.6.5.10.4 ヒト、ラット及びサル肝、腎ミクロソーム並びに肝細胞における代謝安定性（[redacted]RHYTP1）

Metabolic Stability, Non-GLP		**Test Article:** Setmelanotide	
		Location in eCTD: 4.2.2.4-4	
		Study No.: [redacted]RHYTP1	
Study System: In vitro – To determine the metabolic stability in human, rat and monkey liver and kidney microsomes and cryopreserved hepatocytes.			
Liver Microsomal Stability			
Methods:	The reaction mixture minus NADPH, was prepared with liver microsomes, test article or control compound and buffer. An aliquot of the reaction mixture (without cofactor) was equilibrated in a shaking water bath at 37°C for 3 min. The reaction was initiated by the addition of NADPH, and the mixture was incubated in a shaking water bath at 37°C. Aliquots (100 µL) were withdrawn and immediately combined with 400 µL of ice-cold 50/50 acetonitrile /H_2O containing 0.1% formic acid and internal standard to terminate the reaction. The samples were then mixed and centrifuged to precipitate proteins, followed by sample analysis.		
Time:	0, 10, 20, 30, 60 min for RM-493, testosterone		
Concentration:	1 µmol/L for RM-493		
Method of Analysis:	LC-MS/MS		
Test Article	**Species**	**Half-life[a] (min)**	**CL_{int}[b] (mL/min/mg protein)**
RM-493	Human	>60	<0.0231
	Rat	>60	<0.0231
	Monkey	>60	<0.0231

Additional Information: The positive control, testosterone, met all acceptance criteria.

a) When the calculated half-life is longer than the duration of the experiment, the half-life is expressed as > the longest incubation time. Then, if the calculated half-life is < 2× the duration of the experiment, the calculated half-life is listed in parentheses.

b) CL_{int} was calculated based on $CL_{int} = k/P$, where k is the elimination rate constant and P is the protein concentration in the incubation.

Abbreviations: CL_{int} = intrinsic clearance; eCTD = electronic Common Technical Document; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; NADPH = nicotinamide adenine dinucleotide phosphate; RM-493 = setmelanotide.

Metabolic Stability, Non-GLP		Test Article: Setmelanotide	
		Location in eCTD: 4.2.2.4-4	
		Study No.: [redacted]RHYTP1	
Kidney Microsomal Stability			
Methods:	The reaction mixture, minus NADPH, was prepared with kidney microsomes, the test or control articles and buffer. An aliquot of the reaction mixture (without cofactor) was equilibrated in a shaking water bath at 37°C for 3 min. The reaction was initiated by the addition of the cofactor, and the mixture was incubated in a shaking water bath at 37°C. Aliquots (100 µL) were withdrawn and immediately combined with 400 µL of ice-cold 50/50 acetonitrile/H_2O containing 0.1% formic acid and internal standard to terminate the reaction. The samples were then mixed and centrifuged to precipitate proteins, followed by sample analysis.		
Time:	0, 10, 20, 30, 60 min for RM-493, testosterone		
Concentration:	1 µmol/L for RM-493		
Method of Analysis:	LC-MS/MS		
Test Article	**Species**	**Half-life (min)**	**CL_{int} (mL/min/mg/protein)**
RM-493	Human	>60	<0.0231
	Rat	>60	<0.0231
	Monkey	>60	<0.0231
Additional Information: The "positive" control was testosterone, although no metabolic instability was observed and no specifications are reported for acceptance criteria in the literature.			

Abbreviations: CL_{int} = intrinsic clearance; eCTD = electronic Common Technical Document; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; NADPH = nicotinamide adenine dinucleotide phosphate; RM-493 = setmelanotide.

Metabolic Stability, Non-GLP		Test Article: Setmelanotide	
		Location in eCTD: 4.2.2.4-4	
		Study No.: [redacted]RHYTP1	
Cryopreserved Hepatocyte Stability			
Methods:	The hepatocytes were thawed and prepared according to the vendor's instructions, pooled into Krebs Henseleit buffer (pH 7.4), and kept on ice prior to the experiments. The hepatocyte suspension was equilibrated in a shaking water bath at 37°C for 3 min, and then the reaction was initiated by spiking the test article into the hepatocyte suspension (1.5×10^6 cells/mL). The final DMSO content in the incubation mixture was ≤0.1%. The reaction mixture was incubated in a shaking water bath at 37°C. Positive control incubations were performed in parallel to confirm the activity of the hepatocytes. Aliquots were withdrawn and immediately terminated by adding 3 volumes of ice-cold acetonitrile containing internal standard. The samples were then mixed and centrifuged to precipitate proteins.		
Time:	0, 15, 30, 60, 120 min for RM-493 0, 5, 15, 30, 60, 120 min for testosterone 0, 15 min for 7-HC		
Concentration:	1 µmol/L for RM-493, testosterone; 100 µmol/L for 7-HC		
Method of Analysis:	LC-MS/MS		
Test Article	**Species**	**Half-life (min)**	**CL_{int} (mL/min/10^6 cells)**
RM-493	Human	>120	<0.00385
	Rat	>120	<0.00385
	Monkey	>120	<0.00385
Additional Information: The metabolic stability for the positive controls met the acceptance criteria.			

Abbreviations: CL_{int} = intrinsic clearance; DMSO = dimethyl sulfoxide; eCTD = electronic Common Technical Document; GLP = Good Laboratory Practice; 7-HC = 7-hydroxycoumarin; LC-MS/MS = liquid chromatography-tandem mass spectrometry; RM-493 = setmelanotide.

2.6.5.11 In vivo 代謝

2.6.5.11.1 ラットにおける代謝経路（RM-493-PK-008）

Rat Metabolism, Non-GLP	**Test Article**: [^{14}C]-Setmelanotide
	Location in eCTD: 4.2.2.2-1
	Study No.: RM-493-PK-008
Species/Strain:	Rat/Sprague-Dawley
Gender (M/F)/Number of animals:	9M/9F (Intact); 3M/3F (Bile duct Cannulated)
Feeding Condition:	Fed
Vehicle/Formulation:	[^{14}C]-RM-493 in Saline
Method of Administration:	SC / IV
Dose (mg/kg):	2 mg/kg SC; 1 mg/kg IV (100 µCi/kg)
Radionuclide:	^{14}C
Specific Activity:	339 mCi/mmol
Assay:	HPLC with radiometric detection and tandem mass spectrometry
Additional Information:	Metabolic pathways shown below:



Abbreviations: eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; HPLC = high performance liquid chromatography; M = male; IV = intravenous; RM-493 = setmelanotide; SC = subcutaneous.

2.6.5.11.2 ラット尿及び糞中代謝物の同定（RM-493-PK-008）

Rat Metabolism (Excreta), Non-GLP	Test Article: [^{14}C]-Setmelanotide
	Location in eCTD: 4.2.2.2-1
	Study No.: RM-493-PK-008
Species/Strain:	Rat/Sprague-Dawley
Gender (M/F)/Number of animals:	6M/6F (Intact); 3M/3F (Bile Duct Cannulated)
Feeding Condition:	Fed
Vehicle/Formulation:	[^{14}C]-RM-493 in Saline
Method of Administration:	SC / IV
Dose (mg/kg):	2 mg/kg (100 µCi/kg) SC; 1 mg/kg (100 µCi/kg) IV
Radionuclide:	^{14}C
Specific Activity:	339 mCi/mmol
Assay:	HPLC with radiometric detection and tandem mass spectrometry; liquid scintillation counting

			Average % of Dosed Radioactivity[a]											
			Male						Female					
			Urine			Feces			Urine			Feces		
		Group:	SC	IV	SC	SC	IV	SC	SC	IV	SC	SC	IV	SC
$(M+H)^+$	Description	Metabolite/ Intact (I) vs. BDC	I	I	BDC	I	I	BDC	I	I	BDC	I	I	BDC
-	-	M1	1.4	1.8	1.2	ND	ND	ND	1.4	0.9	1.6	ND	ND	ND
-	-	M2	0.2	ND	0.4	ND	ND	ND	0.3	ND	0.1	ND	ND	ND
-	-	M3	0.02	0.18	0.05	ND	ND	ND	ND	0.2	ND	ND	ND	ND
733	-	M4	0.6	0.5	1.1	ND	ND	ND	0.9	0.6	1.6	ND	ND	ND
-	-	M5	1.2	2.5	ND	ND	ND	ND	0.5	2.3	ND	ND	ND	ND
1,007	M19+H_2O-([redacted])	M6	ND	ND	0.3	1.7	0.9	1.6	0.1	ND	1.0	1.8	2.1	0.8
950	M19+H_2O-[redacted]	M7	5.6	ND	3.4	ND	ND	ND	9.8	ND	14.5	ND	ND	ND
1,152	-	M8	ND	ND	ND	ND	ND	ND	0.80	ND	ND	ND	ND	ND
1,113	-	M9	0.08	ND	ND	ND	ND	ND	2.04	ND	ND	ND	ND	ND
1,038	M16-([redacted])	M10	6.0	0.7	2.5	ND	ND	ND	11.2	2.5	1.7	ND	ND	ND
911	-	M11	3.3	ND	1.6	ND	ND	ND	4.6	ND	2.3	ND	ND	ND
1,178	M19+H_2O+[redacted]	M12	9.2	2.2	2.9	ND	ND	ND	11.4	3.4	6.3	ND	ND	ND
-	-	M13	2.0	0.4	ND	ND	ND	ND	1.9	0.5	ND	ND	ND	ND

Rat Metabolism (Excreta), Non-GLP			Test Article: [^{14}C]-Setmelanotide											
			Location in eCTD: 4.2.2.2-1											
			Study No.: RM-493-PK-008											
			Average % of Dosed Radioactivity[a]											
			Male						Female					
			Urine			Feces			Urine			Feces		
		Group:	SC	IV	SC	SC	IV	SC	SC	IV	SC	SC	IV	SC
$(M+H)^+$	Description	Metabolite/ Intact (I) vs. BDC	I	I	BDC	I	I	BDC	I	I	BDC	I	I	BDC
1,135	Hydrolysis	M14	2.8	1.0	2.4	ND	ND	ND	4.0	1.3	2.0	ND	ND	ND
1,135	Hydrolysis	M15	2.2	0.9	1.0	ND	ND	ND	3.3	1.0	1.4	ND	ND	ND
-	-	M16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
830	-	M17	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,117	Setmelanotide	Parent drug	6.8	10.8	24.2	ND	ND	ND	3.8	8.5	29.4	ND	ND	ND
-	-	M18	0.9	0.4	ND	ND	ND	ND	0.1	0.6	ND	ND	ND	ND
1,118	amide hydrolysis	M19	31.1	59.3	24.6	ND	ND	ND	19.1	58.4	14.4	ND	ND	ND
Total	**-**	**-**	**73.3**	**80.9**	**65.6**	**1.7**	**0.9**	**1.6**	**75.3**	**80.4**	**76.3**	**1.83**	**2.1**	**0.8**

Additional Information: Less than 3% of the dosed radioactivity was recovered in bile so this matrix was not profiled for metabolites. Recovery of radioactivity after extraction and/or reconstitution: >95%, urine; 26 – 59%, feces.
Note: Urine Pool = 0 – 48 hr; Feces Pool = 0 – 24 hr;
a) Percent of Dose = ROI × calculated percent of dose represented by the sample pool.

Abbreviations: "-" = unidentified or unknown; BDC = bile-duct cannulated; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; HPLC = high performance liquid chromatography; I = intact; IV = intravenous; M = male; ND = not detected; RM-493 = setmelanotide; ROI = region of interest; SC = subcutaneous.

2.6.5.11.3 ラット血漿中代謝物の同定（RM-493-PK-008）

Rat Metabolism (Plasma), Non-GLP	Test Article: [^{14}C]-Setmelanotide
	Location in eCTD: 4.2.2.2-1
	Study No.: RM-493-PK-008
Species/Strain:	Rat/Sprague-Dawley and Long-Evans
Gender (M/F)/Number of animals:	Groups 1, 2 (9M/9F) and 3 (4M/4F) – Plasma[a]
Feeding Condition:	Fed
Vehicle/Formulation:	[^{14}C]-RM-493 in Saline
Method of Administration:	SC / IV
Dose (mg/kg):	2 mg/kg (SC); 1 mg/kg (IV) (100 µCi/kg)
Radionuclide:	^{14}C
Specific Activity:	339 mCi/mmol
Assay:	HPLC with radiometric detection and tandem mass spectrometry

$(M + H)^+$	Description	Metabolite ID	Male		Female		Pool 1	Pool 2
			SC	IV	SC	IV	Group 3	Group 3
			% ROI	% ROI	% ROI	% ROI	% ROI	% ROI
-	-	M1	ND	ND	ND	ND	ND	ND
-	-	M2	ND	ND	ND	ND	ND	ND
-	-	M3	ND	ND	ND	ND	ND	ND
733	-	M4	ND	ND	ND	ND	ND	ND
-	-	M5	ND	ND	ND	ND	ND	ND
1,007	M19+H_2O-([redacted])	M6	ND	ND	ND	ND	ND	ND
950	M19+H_2O-[redacted]	M7	ND	ND	ND	ND	ND	ND
1,152	-	M8	ND	ND	ND	ND	ND	ND
1,113	-	M9	ND	ND	ND	ND	ND	ND
1,038	M16-([redacted])	M10	ND	ND	ND	ND	ND	ND
911	-	M11	ND	ND	ND	ND	ND	ND
1,178	M19+H_2O+[redacted]	M12	ND	ND	ND	ND	ND	ND
-	-	M13	ND	ND	ND	ND	ND	ND
1,135	Hydrolysis	M14	ND	ND	ND	ND	ND	ND
1,135	Hydrolysis	M15	ND	ND	ND	ND	ND	ND
-	-	M16	6.5	0.4	4.5	0.9	7.1	4.9
830	-	M17	3.5	0.8	3.6	0.1	5.6	5.1

Rat Metabolism (Plasma), Non-GLP			Test Article: [^{14}C]-Setmelanotide					
			Location in eCTD: 4.2.2.2-1					
			Study No.: RM-493-PK-008					
$(M + H)^+$	Description	Metabolite ID	Male		Female		Pool 1	Pool 2
			SC	IV	SC	IV	Group 3	Group 3
			% ROI	% ROI	% ROI	% ROI	% ROI	% ROI
1,117	Setmelanotide	Parent	85.0	94.6	89.1	91.3	75.7	79.2
-	-	M18	ND	ND	ND	ND	ND	ND
1,118	amide hydrolysis	M19	5.0	4.2	2.9	7.6	11.6	10.8

Additional Information: "-" = Unknown or Unidentified; % ROI = percent region of interest. Radioactive recoveries in rat plasma are likely incomplete and/or skewed relative to the pharmacokinetic parameters in Section 2.6.5.3.1 that suggest that circulating radioactivity is 3- to 5-fold higher than unchanged RM-493 in circulation.

a) Plasma Pools: G1: 0.5 – 2 hr; G2: 5 min – 1 hr; G3: 0.5 – 3 hr.

Abbreviations: eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; HPLC = high performance liquid chromatography; IV = intravenous; M = male; ND = not detected; RM-493 = setmelanotide; SC = subcutaneous.

2.6.5.12 薬物動態：排泄

2.6.5.12.1 ラットにおける尿、胆汁及び糞中排泄（RM-493-PK-008）

Rat Excretion, Non-GLP	**Test Article:** [^{14}C]-Setmelanotide									
	Location in eCTD: 4.2.2.2-1									
	Study No.: RM-493-PK-008									
Species/Strain:	**Rat/Sprague-Dawley** (Intact)					**Rat/Sprague-Dawley** (Intact)				
Gender (M/F)/Number of Animals:	3M					3F				
Feeding Condition:	Fed					Fed				
Vehicle/Formulation:	RM-493 in Saline					RM-493 in Saline				
Method of Administration:	SC					SC				
Dose (mg/kg):	2					2				
Analyte:	^{14}C					^{14}C				
Assay:	LSC[c]					LSC[c]				
Excretion Route:	**Average % of Dose Recovered (Cumulative)**					**Average % of Dose Recovered (Cumulative)**				
Time	**Urine**	**Feces**	**Cage Rinse**	**Cage Wash**	**Total**	**Urine**	**Feces**	**Cage Rinse**	**Cage Wash**	**Total**
0 – 6 hr	66.3	ND	9.22	ND	**75.5**	66.4	ND	6.84	ND	**73.2**
0 – 12 hr	70.1	0.34[a]	10.1	ND	**80.5**	71.6	0.13[a]	7.72	ND	**79.5**
0 – 24 hr	72.0	4.78	10.6	ND	**87.4**	73.6	1.94	8.45	ND	**84.0**
0 – 48 hr	73.3	5.90	10.7	ND	**89.9**	75.3	4.62	8.61	ND	**88.5**
0 – 72 hr	73.7	6.27	10.9	ND	**90.9**	75.7	5.32	8.72	ND	**89.7**
0 – 96 hr	73.9	6.43	10.9	ND	**91.2**	75.9	5.49	8.76	ND	**90.2**
0 – 120 hr	74.1	6.55	11.0	ND	**91.7**	76.1	5.65	8.79	ND	**90.5**
0 – 144 hr	74.2	6.61	11.0	ND	**91.8**	76.1	5.71	8.83	ND	**90.6**
0 – 168 hr	74.2	6.65	11.0	0.06[b]	**91.9**	76.2	5.76	8.84	0.03[b]	**90.8**
Total	**74.2**	**6.65**	**11.0**	**0.06**	91.9	**76.2**	**5.76**	**8.84**	**0.03**	90.8

Additional Information:
a) Time period of 0-12 hours for feces.
b) 168 hours (end of study) only for cage wash.
c) Fecal homogenate samples were combusted prior to LSC.

Abbreviations: eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LSC = liquid scintillation counting; M = male; ND = not determined; RM-493 = setmelanotide; SC = subcutaneous.

Rat Excretion, Non-GLP	**Test Article:** [^{14}C]-Setmelanotide									
	Location in eCTD: 4.2.2.2-1									
	Study No.: RM-493-PK-008									
Species/Strain:	**Rat/Sprague-Dawley** (Intact)					**Rat/Sprague-Dawley** (Intact)				
Gender (M/F)/Number of Animals:	3M					3F				
Feeding Condition:	Fed					Fed				
Vehicle/Formulation:	RM-493 in Saline					RM-493 in Saline				
Method of Administration:	IV					IV				
Dose (mg/kg):	1					1				
Analyte:	^{14}C					^{14}C				
Assay:	LSC[c]					LSC[c]				
Excretion Route:	**Average % of Dose Recovered (Cumulative)**					**Average % of Dose Recovered (Cumulative)**				
Time	**Urine**	**Feces**	**Cage Rinse**	**Cage Wash**	**Total**	**Urine**	**Feces**	**Cage Rinse**	**Cage Wash**	**Total**
0 – 6 hr	72.8	ND	11.8	ND	**84.6**	74.6	ND	4.82	ND	**79.4**
0 – 12 hr	77.0	1.33[a]	12.7	ND	**91.0**	77.2	0.04[a]	5.48	ND	**82.7**
0 – 24 hr	79.4	3.84	13.4	ND	**96.6**	79.3	5.54	5.77	ND	**90.6**
0 – 48 hr	80.9	5.16	13.5	ND	**99.6**	80.4	7.23	5.92	ND	**93.6**
0 – 72 hr	81.3	5.53	13.7	ND	**100.5**	80.8	7.57	5.99	ND	**94.4**
0 – 96 hr	81.6	5.69	13.7	ND	**101.0**	81.0	7.76	6.06	ND	**94.8**
0 – 120 hr	81.7	5.80	13.8	ND	**101.3**	81.1	7.88	6.10	ND	**95.1**
0 – 144 hr	81.8	5.87	13.8	ND	**101.5**	81.2	7.95	6.13	ND	**95.3**
0 – 168 hr	81.9	5.94	13.8	0.05[b]	**101.7**	81.3	8.00	6.14	0.04[b]	**95.5**
Total	**81.9**	**5.94**	**13.8**	**0.05**	**101.7**	**81.3**	**8.00**	**6.14**	**0.04**	**95.5**

Additional Information:
a) Time period of 0-12 hours for feces.
b) 168 hours (end of study) only for cage wash.
c) Fecal homogenate samples were combusted prior to LSC.

Abbreviations: eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; IV = intravenous; LSC = liquid scintillation counting; M = male; ND = not determined; RM-493 = setmelanotide.

Rat Excretion, Non-GLP	**Test Article:** [^{14}C]-Setmelanotide											
	Location in eCTD: 4.2.2.2-1											
	Study No.: RM-493-PK-008											
Species/Strain:	**Rat/Sprague-Dawley** (Bile Duct Cannulated)						**Rat/Sprague-Dawley** (Bile Duct Cannulated)					
Gender (M/F)/Number of Animals:	3M						3F					
Feeding Condition:	Fed						Fed					
Vehicle/Formulation:	RM-493 in Saline						RM-493 in Saline					
Method of Administration:	SC						SC					
Dose (mg/kg):	2						2					
Analyte:	^{14}C						^{14}C					
Assay:	LSC[c]						LSC[c]					
Excretion Route:	**Average % of Dose Recovered**						**Average % of Dose Recovered**					
Time	**Urine**	**Bile**	**Feces**	**Cage Rinse**	**Cage Wash**	**Total**	**Urine**	**Bile**	**Feces**	**Cage Rinse**	**Cage Wash**	**Total**
0 – 4 hr	42.0	1.02	ND	11.6	ND	**54.6**	61.8	1.36	ND	7.87	ND	**71.0**
4 – 8 hr	18.2	0.39	0.34[a]	1.43	ND	**20.4**	9.77	0.38	0.26[a]	1.33	ND	**11.7**
8 – 24 hr	3.58	0.21	0.71	0.35	ND	**4.85**	3.28	0.21	0.08	0.53	ND	**4.10**
24 – 48 hr	1.82	0.09	5.91	0.16	0.28[b]	**8.26**	1.53	0.10	1.31	0.23	0.32[b]	**3.49**
Total	**65.6**	**1.70**	**6.95**	**13.5**	**0.28**	**88.1**	**76.3**	**2.05**	**1.64**	**9.96**	**0.32**	**90.3**

Additional Information:
a) Time period of 0-8 hours for feces.
b) 48 hours (end of study) only for cage wash.
c) Fecal homogenate samples were combusted prior to LSC.

Abbreviations: eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LSC = liquid scintillation counting; M = male; ND = not determined; RM-493 = setmelanotide; SC = subcutaneous.

2.6.5.12.2 ラットの妊娠又は授乳動物における乳汁中排泄（RM-493-TOX-033）

Rat Excretion (Milk), GLP	**Test Article:** Setmelanotide		
	Location in eCTD: 4.2.3.5.3		
	Study No.: RM-493-TOX-033		
Species/Strain:	Rat/Sprague-Dawley		
Lactation day/Number of Animals:	Day 11: 3 – 4 F		
Vehicle/Formulation:	mPEG-DSPE Diluted 1:10 in 5% Mannitol		
Method of Administration:	SC Injection		
Dose (mg/kg/day):	0.5, 3.0, 5.0 from Day 6 pc to Lactation Day 21		
Sample (e.g., whole blood, plasma, serum):	dam and pup plasma, dam milk		
Analyte:	RM-493 in dam and pup plasma and dam milk		
Assay:	LC-MS/MS		
Dose (mg/kg)	**0.5**	**3**	**5**
Time (hr) on Day 11 pp	**2**	**2**	**2**
Concentration/Amount (ng/mL)			
Milk:	12.0	83.0	106
Plasma:	30.7	393	636
Milk/Plasma:	0.39	0.21	0.17
Neonates:	BLQ	BLQ	BLQ

Abbreviations: BLQ = below the limit of quantitation; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; mPEG-DSPE = N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; pc = post-coitum; pp = post-partum; RM-493 = setmelanotide; SC = subcutaneous.

2.6.5.13 薬物動態学的相互作用試験

2.6.5.13.1 In vitro チトクローム P450 に対する阻害試験（T0063）

CYP inhibition, Non-GLP	**Test Article:** Setmelanotide
	Location in eCTD: 4.2.2.6-1
Type of Study: In Vitro	**Study No.:** T0063

Study System: In vitro – The inhibition potential of RM-493 was evaluated with the recombinant cytochrome P450s (CYPs) 1A2, 2C9, 2C19, 2D6, and 3A4. Incubations were conducted in a 200 µL volume in 96 well microtiter plates. A cofactor/deionized water mix was prepared that contained the NADPH regenerating system and control insect cell membrane protein. To the first well in each row, 150 µL of cofactor/ deionized water mix containing the upper concentration of inhibitor was added, and a serial dilution was performed in the next wells. Wells 9 and 10 contained no test article (total enzyme activity) and wells 11 and 12 were used as controls for background fluorescence (non-specific activity). The plate was then pre-incubated at 37°C for 10 min and the reaction initiated by the addition of 100 µL of pre-warmed enzyme/substrate (E/S) mix; the E/S mix contained buffer, cDNA-expressed CYPs in insect cell microsomes, and the appropriate substrate(s). The enzyme/substrate stock concentration was twice the final concentration (final substrate concentration was the apparent K_m). Reactions were terminated after a suitable incubation period by addition of 75 µL of acetonitrile (4°C), 0.5 mol/L Tris base solution. Fluorescence was measured using an EnVision® Multilabel plate reader.

Time:	10 min
Method of Analysis:	Fluorescence

IC_{50} (µmol/L) and 95% Confidence Interval of RM-493 and Reference Inhibitors

CYP	RM-493 (µmol/L)	Reference Inhibitor (µmol/L)	BD-Gentest Values (µmol/L)	Reference Inhibitor
1A2/CEC	>1,000	1.21 [0.855 – 1.68]	2.11 – 1.3	Furafylline
2C9/MFC	>1,000	0.36 [0.2 – 0.651]	0.51 – 0.27	Sulfaphenazole
2C19/CEC	>1,000	2.45 [0.68 – 9.87]	4	Tranylcypromine
2D6/AMMC	>1,000	0.0037 [0.0028 – 0.0048]	0.005 – 0.014	Quinidine
3A4/BQ	76.2 [20 - 482]	0.026 [0.013 – 0.054]	0.072/0.4	Ketoconazole
3A4/BFC	54.1 [33.6 – 88.0]	0.039 [0.012 – 0.163]	0.034/0.005	Ketoconazole

Additional Information:
Note: Brackets indicate the 95% confidence interval. See Sections 2.6.5.13.4, 2.6.5.13.5, and 2.6.5.13.6 for additional CYP assessments.

Abbreviations: AMMC = 3-[2-(*N*,*N*-diethyl-*N*-methylammonium)ethyl]-7-methoxy-4-methylcoumarin; BFC = 7-benzyloxy-4-(trifluoromethyl)-coumarin; BQ = 7-benzyloxyquinoline; cDNA = complementary deoxyribonucleic acid; CEC = 3-cyano-7-ethoxycoumarin; CYP = cytochrome P450; eCTD = electronic Common Technical Document; GLP = Good Laboratory Practice; IC_{50} = 50% inhibitory concentration; K_m = Michaelis-Menten constant; MFC = 7-methoxy-4-(trifluoromethyl)-coumarin; NADPH = nicotinamide adenine dinucleotide phosphate; RM-493 = setmelanotide.

2.6.5.13.2 ヒト肝ミクロソームを用いたチトクローム P450 に対する阻害試験（[redacted]RHYTP2_CYP Inhibition）

CYP inhibition, Non-GLP	**Test Article:** Setmelanotide
	Location in eCTD: 4.2.2.6-2
	Study No.: [redacted]RHYTP2_CYP Inhibition

Study System: In vitro – The test article (1.2 µmol/L) was incubated with pooled HLM (0.25 mg protein/mL) in phosphate buffer (100 mmol/L, pH 7.4) containing $MgCl_2$ (5 mmol/L), CYP-specific probe substrate (at approximately K_m), and NADPH (1 mmol/L). After a period of incubation, the reaction was terminated by the addition of ice-cold protein precipitation solvent (acetonitrile containing an internal standard) and the samples were centrifuged. CYP enzyme activities were measured by determining the formation of CYP probe metabolites by LC-MS/MS, and the percent inhibition was estimated in comparison with the vehicle controls. Known CYP inhibitors were run in parallel as positive controls.

Inhibition of CYP Activities in HLM by RM-493

CYP	Probe Substrate	Metabolite	% Inhibition (n=3) Average (SD)[a]
CYP1A2	Phenacetin	Acetaminophen	0 (4.9)
CYP2A6	Coumarin	7-OH coumarin	0 (0.62)
CYP2B6	Bupropion	OH bupropion	0 (3.2)
CYP2C8	Amodiaquine	Desethylamodiaquine	0 (2.6)
CYP2C9	Diclofenac	4'-OH diclofenac	0 (3.6)
CYP2C19	S-mephenytoin	4'-OH mephenytoin	0 (2.4)
CYP2D6	Bufuralol	1'-OH bufuralol	0 (0.44)
CYP2E1	Chlorzoxazone	6-OH chlorzoxazone	0 (3.6)
CYP3A	Midazolam	1'-OH midazolam	18.2 (2.0)
	Testosterone	6β-OH testosterone	10.8 (2.3)

Additional Information: The results suggest that RM-493 at 1.2 µmol/L has weak inhibition of CYP3A and no inhibition of the other major CYPs tested.
a) Negative values of % inhibition are treated as zero.

Abbreviations: CYP = cytochrome P450; eCTD = electronic Common Technical Document; GLP = Good Laboratory Practice; HLM = human liver microsomes; K_m = Michaelis-Menten constant; LC-MS/MS = liquid chromatography-tandem mass spectrometry; NADPH = nicotinamide adenine dinucleotide phosphate; RM-493 = setmelanotide; SD = standard deviation.

2.6.5.13.3 ヒト肝ミクロソームを用いたチトクローム P450 に対する時間依存的阻害試験（[redacted]RHYTP2_CYP TDI）

CYP time-dependent inhibition, Non-GLP	Test Article: Setmelanotide
	Location in eCTD: 4.2.2.6-3
	Study No.: [redacted]RHYTP2_CYP TDI

Study System: In vitro – The test article, at eight concentrations (0-2.4 µmol/L), was pre-incubated with pooled HLMs (0.25 mg protein/mL) for 30 min in the presence and absence of NADPH, followed by a CYP enzyme activity assay with an individual CYP probe substrate and quantification by LC-MS/MS. The IC_{50} was estimated by non-linear regression analysis.

CYP	Pre-incubation (30 min)	% of Control Enzyme Activity (average, n=2)[a]								IC_{50}[b] (µmol/L)	IC_{50} Shift[c]	TDI[d]
		0 µmol/L	0.0033 µmol/L	0.0099 µmol/L	0.030 µmol/L	0.089 µmol/L	0.27 µmol/L	0.80 µmol/L	2.4 µmol/L			
CYP1A2	−NADPH	100	107	109	101	105	111	105	95.2	>2.4	ND	Unlikely
	+NADPH	100	101	101	94.8	95.4	98.2	97.8	91.8	>2.4		
CYP2A6	−NADPH	100	98.2	96.9	96.1	95.6	98.2	97.2	99.2	>2.4	ND	Unlikely
	+NADPH	100	106	104	110	107	109	114	118	ND		
CYP2B6	−NADPH	100	101	106	101	106	100	104	96.3	>2.4	ND	Unlikely
	+NADPH	100	95.1	98.4	104	99.2	100	107	96.3	>2.4		
CYP2C8	−NADPH	100	106[e]	88.2	89.5	89.2	90.1	91.8	92.2	>2.4	ND	Unlikely
	+NADPH	100	---	100	97.7	92.0	103	108	105	ND		
CYP2C9	−NADPH	100	97.0	94.3	92.4	86.6	88.0	94.2	93.5	>2.4	ND	Unlikely
	+NADPH	100	95.8	86.6	87.4	88.1	91.5	92.0	95.8	>2.4		
CYP2C19	−NADPH	100	92.3	97.7	92.4	95.4	100	109	106	ND	ND	Unlikely
	+NADPH	100	89.0	86.5	87.1	91.8	89.6	83.6	83.8	>2.4		
CYP2D6	−NADPH	100	94.3	89.9	92.3	95.2	103	99.0	103	ND	ND	Unlikely
	+NADPH	100	106	105	99.3	104	105	104	106	ND		
CYP2E1	−NADPH	100	107	100	101	99.0	99.0	105	105	ND	ND	Unlikely
	+NADPH	100	101	108	97.6	101	108	111	101	ND		
CYP3A (Midazolam)	−NADPH	100	106	97.4	103	98.2	96.6	87.3	74.2	>2.4	ND	Unlikely
	+NADPH	100	98.0	94.9	95.3	94.2	94.8	88.3	81.6	>2.4		
CYP3A (Testosterone)	−NADPH	100	102	104	96.8	99.1	94.8	88.5	63.7	>2.4	ND	Unlikely
	+NADPH	100	104	113	109	102	108	103	88.2	>2.4		

Additional Information: The results suggest that RM-493 is unlikely to be a time-dependent inhibitor of major hepatic CYPs.

a) Percent of control enzyme activity = 100 × (enzyme activity in the presence of TA/enzyme activity in the absence of TA). Enzyme activity was calculated from the peak area ratio of CYP probe metabolite to IS by LC-MS/MS.
b) When the enzyme activity was >50% of control at the highest TA concentration, the IC_{50} is expressed as > the highest concentration.
c) IC_{50} shift = $IC_{50\ (-NADPH)}$ / $IC_{50\ (+NADPH)}$.
d) IC_{50} shift >1.5, TDI likely.
e) n=1.

Note: ND indicates not detectable due to no inhibition up to 2.4 µmol/L (the highest concentration tested in this study).

Abbreviations: CYP = cytochrome P450; eCTD = electronic Common Technical Document; GLP = Good Laboratory Practice; HLM = human liver microsomes; IC_{50} = 50% inhibitory concentration; IS = internal standard; LC-MS/MS = liquid chromatography-tandem mass spectrometry; NADPH = nicotinamide adenine dinucleotide phosphate; ND = not detectable; RM-493 = setmelanotide; TA = test article; TDI = time-dependent inhibition.

2.6.5.13.4 ヒト肝細胞を用いたチトクローム P450 に対する誘導試験（[redacted]RHYTP2_CYP Induction）

CYP induction, Non-GLP	Test Article: Setmelanotide
	Location in eCTD: 4.2.2.6-4
	Study No.: [redacted]RHYTP2_CYP Induction

Study System: In vitro – Plateable and inducible cryopreserved human hepatocytes were treated with RM-493 at various concentrations at 37ºC for three days (72 hours) with daily replacement of the cell culture media containing the test article, positive control, or vehicle. After induction treatment, CYP mRNA expression was measured by qPCR and CYP enzyme activity was measured by determining the formation of CYP probe metabolite by LC-MS/MS. CYP induction was evaluated with fold-increase of mRNA expression and enzyme activity. Cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium, inner salt (MTS) assay and cytotoxicity was evaluated by the percentage of MTS absorbance relative to the vehicle control.

Cytotoxicity

		Cell Viability after Treatment with RM-493 or Positive Control		
Test Article	Treatment	Formazan Absorbance (n=3)		%MTS Absorbance Relative to Vehicle Control[a]
		Mean	SD	
Control	Vehicle	1.28	0.18	100
	Chlorpromazine	0.488	0.10	38
RM-493	0.01 µmol/L	1.14	0.13	90
	0.05 µmol/L	1.24	0.29	97
	0.1 µmol/L	1.29	0.22	101
	0.5 µmol/L	1.36	0.13	107
	1 µmol/L	1.64	0.25	128

a) MTS absorbance <75% of the vehicle control is considered a positive cytotoxic result. QC acceptance criterion: % MTS absorbance relative to the vehicle control ≤50 by positive control.

CYP induction, Non-GLP			Test Article: Setmelanotide		
			Location in eCTD: 4.2.2.6-4		
			Study No.: [redacted]RHYTP2_CYP Induction		
Induction of CYP1A2 mRNA					
Donor	**Test Article**	**Treatment**	**Fold-Increase[a] (n=3)**		**% Induction Relative to Positive Control[b]**
			Mean	**SD**	
1	Control	Vehicle	1.02	0.25	0
		OME	67.4	4.2	100
		Flumazenil	1.83	0.29	1.2
	RM-493	0.05 µmol/L	1.48	0.39	0.69
		0.2 µmol/L	1.88	0.20	1.3
		0.5 µmol/L	2.38	1.2	2.0
2	Control	Vehicle	1.00	0.069	0
		OME	17.1	2.3	100
		Flumazenil	0.921	0.20	0
	RM-493	0.05 µmol/L	0.473	0.17	0
		0.2 µmol/L	0.684	0.16	0
		0.5 µmol/L	0.356	0.052	0
3	Control	Vehicle	1.02	0.28	0
		OME	33.1	5.8	100
		Flumazenil	0.909	0.19	0
	RM-493	0.05 µmol/L	0.720	0.31	0
		0.2 µmol/L	0.828	0.11	0
		0.5 µmol/L	0.959	0.14	0

Additional Information:

a) Fold-increase was calculated from the normalized mRNA level ($2^{-\Delta\Delta Ct}$) of TA-, positive inducer-, or negative inducer-treated cells relative to that of vehicle-treated cells.

b) Percentage of mRNA fold-increase relative to positive inducer-treated cells. Negative values are reported as zero.

CYP induction, Non-GLP			Test Article: Setmelanotide			
			Location in eCTD: 4.2.2.6-4			
			Study No.: [redacted]RHYTP2_CYP Induction			
Induction of CYP1A2 Enzyme Activity						
Donor	**Test Article**	**Treatment**	**Acetaminophen Formation (pmol/well/min, n=3)**		**Fold-Increase[a]**	**%Induction Relative to Positive Control[b]**
			Mean	**SD**		
1	Control	Vehicle	2.30	0.37	1.0	0
		OME	292	24	127	100
		Flumazenil	1.94	0.19	0.84	0
	RM-493	0.05 µmol/L	1.64	0.44	0.71	0
		0.2 µmol/L	2.19	0.31	0.95	0
		0.5 µmol/L	1.80	0.25	0.78	0
2	Control	Vehicle	3.73	0.39	1.0	0
		OME	279	49	75	100
		Flumazenil	3.38	0.74	0.91	0
	RM-493	0.05 µmol/L	2.06	0.21	0.55	0
		0.2 µmol/L	2.97	0.19	0.80	0
		0.5 µmol/L	2.75	0.23	0.74	0
3	Control	Vehicle	1.08	0.22	1.0	0
		OME	31.5	2.6	29	100
		Flumazenil	0.910	0.22	0.85	0
	RM-493	0.05 µmol/L	0.501	0.13	0.47	0
		0.2 µmol/L	0.810	0.052	0.75	0
		0.5 µmol/L	0.758	0.13	0.70	0

Additional Information:

a) Fold-increase was calculated from the ratio of normalized enzyme activity (formation rate of probe substrate metabolite) of TA-, positive inducer-, or negative inducer-treated cells to that of vehicle-treated cells.

b) Percentage of enzyme activity fold-increase relative to positive inducer-treated cells. Negative values are reported as zero.

CYP induction, Non-GLP			Test Article: Setmelanotide		
			Location in eCTD: 4.2.2.6-4		
			Study No.: [redacted]RHYTP2_CYP Induction		
Cell Viability after CYP1A2 Induction Treatment					
Donor	Test Article	Treatment	Formazan Absorbance (n=3)		% MTS Absorbance Relative to Vehicle Control
			Mean	SD	
1	Control	Vehicle	0.454	0.045	100
		OME	0.409	0.010	90
		Flumazenil	0.436	0.017	96
	RM-493	0.05 µmol/L	0.423	0.11	93
		0.2 µmol/L	0.372	0.030	82
		0.5 µmol/L	0.372	0.015	82
2	Control	Vehicle	0.496	0.037	100
		OME	0.446	0.065	90
		Flumazenil	0.506	0.090	102
	RM-493	0.05 µmol/L	0.489	0.026	99
		0.2 µmol/L	0.483	0.049	97
		0.5 µmol/L	0.469	0.023	94
3	Control	Vehicle	0.464	0.043	100
		OME	0.468	0.031	101
		Flumazenil	0.430	0.026	93
	RM-493	0.05 µmol/L	0.413	0.045	89
		0.2 µmol/L	0.432	0.028	93
		0.5 µmol/L	0.425	0.040	92

CYP induction, Non-GLP			Test Article: Setmelanotide		
			Location in eCTD: 4.2.2.6-4		
			Study No.: [redacted]RHYTP2_CYP Induction		
Induction of CYP2B6 mRNA					
Donor	**Test Article**	**Treatment**	**Fold-Increase[a] (n=3)**		**% MTS Absorbance Relative to Vehicle Control[b]**
			Mean	**SD**	
1	Control	Vehicle	1.02	0.23	0
		PB	48.2	8.7	100
		Flumazenil	1.74	0.37	1.5
	RM-493	0.05 µmol/L	1.71	0.51	1.5
		0.2 µmol/L	1.97	0.77	2.0
		0.5 µmol/L	1.54	0.11	1.1
2	Control	Vehicle	1.01	0.16	0
		PB	13.6	1.5	100
		Flumazenil	1.17	0.12	1.3
	RM-493	0.05 µmol/L	0.934	0.53	0
		0.2 µmol/L	0.885	0.15	0
		0.5 µmol/L	0.609	0.12	0
3	Control	Vehicle	1.04	0.33	0
		PB	16.6	2.8	100
		Flumazenil	1.48	0.29	2.9
	RM-493	0.05 µmol/L	1.25	0.31	1.4
		0.2 µmol/L	1.00	0.26	0
		0.5 µmol/L	1.09	0.13	0.33

Additional Information:

a) Fold-increase was calculated from the normalized mRNA level ($2^{-\Delta\Delta Ct}$) of TA-, positive inducer-, or negative inducer-treated cells relative to that of vehicle-treated cells.

b) Percentage of mRNA fold-increase relative to positive inducer-treated cells. Negative values are reported as zero.

CYP induction, Non-GLP			Test Article: Setmelanotide			
			Location in eCTD: 4.2.2.6-4			
			Study No.: [redacted]RHYTP2_CYP Induction			
Induction of CYP2B6 Enzyme Activity						
Donor	**Test Article**	**Treatment**	**OH Bupropion Formation (pmol/well/min, n=3)**		**Fold-Increase[a]**	**%Induction Relative to Positive Control[b]**
			Mean	**SD**		
1	Control	Vehicle	3.02	0.36	1.0	0
		PB	18.8	1.7	6.2	100
		Flumazenil	2.93	0.25	0.97	0
	RM-493	0.05 µmol/L	2.48	0.35	0.82	0
		0.2 µmol/L	2.80	0.045	0.93	0
		0.5 µmol/L	2.88	0.26	0.95	0
2	Control	Vehicle	8.35	1.2	1.0	0
		PB	48.3	1.5	5.8	100
		Flumazenil	8.87	0.56	1.1	1.3
	RM-493	0.05 µmol/L	7.34	0.56	0.88	0
		0.2 µmol/L	7.59	0.17	0.91	0
		0.5 µmol/L	8.28	0.64	0.99	0
3	Control	Vehicle	1.37	0.33	1.0	0
		PB	12.5	1.0	9.2	100
		Flumazenil	1.27	0.13	0.93	0
	RM-493	0.05 µmol/L	1.03	0.25	0.76	0
		0.2 µmol/L	1.31	0.31	0.96	0
		0.5 µmol/L	1.27	0.29	0.93	0

Additional Information:

a) Fold-increase was calculated from the ratio of normalized enzyme activity (formation rate of probe substrate metabolite) of TA-, positive inducer-, or negative inducer-treated cells to that of vehicle-treated cells.

b) Percentage of enzyme activity fold-increase relative to positive inducer-treated cells. Negative values are reported as zero.

CYP induction, Non-GLP			Test Article: Setmelanotide		
			Location in eCTD: 4.2.2.6-4		
			Study No.: [redacted]RHYTP2_CYP Induction		
Cell Viability after CYP2B6 Induction Treatment					
Donor	Test Article	Treatment	Formazan Absorbance (n=3)		% MTS Absorbance Relative to Vehicle Control
			Mean	SD	
1	Control	Vehicle	0.399	0.00058	100
		PB	0.464	0.029	116
		Flumazenil	0.408	0.015	102
	RM-493	0.05 µmol/L	0.410	0.074	103
		0.2 µmol/L	0.381	0.011	95
		0.5 µmol/L	0.380	0.0060	95
2	Control	Vehicle	0.498	0.078	100
		PB	0.409	0.010	82
		Flumazenil	0.497	0.0057	100
	RM-493	0.05 µmol/L	0.474	0.059	95
		0.2 µmol/L	0.494	0.023	99
		0.5 µmol/L	0.444	0.026	89
3	Control	Vehicle	0.446	0.029	100
		PB	0.418	0.037	94
		Flumazenil	0.438	0.032	98
	RM-493	0.05 µmol/L	0.431	0.057	96
		0.2 µmol/L	0.394	0.034	88
		0.5 µmol/L	0.441	0.024	99

CYP induction, Non-GLP			Test Article: Setmelanotide		
			Location in eCTD: 4.2.2.6-4		
			Study No.: [redacted]RHYTP2_CYP Induction		
Induction of CYP3A4 mRNA					
Donor	**Test Article**	**Treatment**	**Fold-Increase[a] (n=3)**		**%Induction Relative to Positive Control[b]**
			Mean	**SD**	
1	Control	Vehicle	1.01	0.16	0
		RIF	13.4	0.19	100
		Flumazenil	1.23	0.32	1.8
	RM-493	0.05 µmol/L	1.37	0.18	2.9
		0.2 µmol/L	1.45	0.20	3.5
		0.5 µmol/L	1.38	0.10	3.0
2	Control	Vehicle	1.00	0.12	0
		RIF	5.89	0.57	100
		Flumazenil	0.945	0.15	0
	RM-493	0.05 µmol/L	1.07	0.057	1.3
		0.2 µmol/L	1.29	0.20	5.8
		0.5 µmol/L	1.01	0.14	0.18
3	Control	Vehicle	1.01	0.21	0
		RIF	12.5	0.75	100
		Flumazenil	1.05	0.10	0.28
	RM-493	0.05 µmol/L	0.864	0.064	0
		0.2 µmol/L	1.00	0.12	0
		0.5 µmol/L	1.10	0.10	0.73

Additional Information:

a) Fold-increase was calculated from the normalized mRNA level ($2^{-\Delta\Delta Ct}$) of TA-, positive inducer-, or negative inducer-treated cells relative to that of vehicle-treated cells.

b) Percentage of mRNA fold-increase relative to positive inducer-treated cells. Negative values are reported as zero.

CYP induction, Non-GLP			Test Article: Setmelanotide			
			Location in eCTD: 4.2.2.6-4			
			Study No.: [redacted]RHYTP2_CYP Induction			
Induction of CYP3A Enzyme Activity						
Donor	**Test Article**	**Treatment**	**1'-OH Midazolam Formation (pmol/well/min, n=3)**		**Fold-Increase[a]**	**%Induction Relative to Positive Control[b]**
			Mean	**SD**		
1	Control	Vehicle	2.39	0.59	1.0	0
		RIF	22.1	5.0	9.3	100
		Flumazenil	1.89	0.31	0.79	0
	RM-493	0.05 µmol/L	2.32	0.49	0.97	0
		0.2 µmol/L	2.47	0.38	1.0	0.43
		0.5 µmol/L	2.29	0.24	0.96	0
2	Control	Vehicle	3.83	0.33	1.0	0
		RIF	31.8	6.2	8.3	100
		Flumazenil	3.61	0.54	0.94	0
	RM-493	0.05 µmol/L	4.03	1.0	1.1	0.71
		0.2 µmol/L	4.70	1.5	1.2	3.1
		0.5 µmol/L	4.93	0.70	1.3	3.9
3	Control	Vehicle	1.95	0.40	1.0	0
		RIF	11.1	0.51	5.7	100
		Flumazenil	2.45	0.51	1.3	5.5
	RM-493	0.05 µmol/L	2.30	0.21	1.2	3.9
		0.2 µmol/L	2.25	0.29	1.2	3.3
		0.5 µmol/L	2.59	0.25	1.3	7.1

Additional Information:

a) Fold-increase was calculated from the ratio of normalized enzyme activity (formation rate of probe substrate metabolite) of TA-, positive inducer-, or negative inducer-treated cells to that of vehicle-treated cells.

b) Percentage of enzyme activity fold-increase relative positive inducer-treated cells. Negative values are reported as zero.

CYP induction, Non-GLP			Test Article: Setmelanotide		
			Location in eCTD: 4.2.2.6-4		
			Study No.: [redacted]RHYTP2_CYP Induction		
Cell Viability after CYP3A Induction Treatment					
Donor	**Test Article**	**Treatment**	**Formazan Absorbance (n=3)**		**% MTS Absorbance Relative to Vehicle Control**
			Mean	**SD**	
1	Control	Vehicle	0.466	0.035	100
		RIF	0.586	0.10	126
		Flumazenil	0.455	0.024	98
	RM-493	0.05 µmol/L	0.489	0.020	105
		0.2 µmol/L	0.498	0.022	107
		0.5 µmol/L	0.488	0.018	105
2	Control	Vehicle	0.416	0.034	100
		RIF	0.363	0.091	87
		Flumazenil	0.452	0.047	109
	RM-493	0.05 µmol/L	0.383	0.077	92
		0.2 µmol/L	0.350	0.096	84
		0.5 µmol/L	0.327	ND	78
3	Control	Vehicle	0.611	0.076	100
		RIF	0.754	0.049	123
		Flumazenil	0.518	0.097	85
	RM-493	0.05 µmol/L	0.521	0.040	85
		0.2 µmol/L	0.504	0.091	82
		0.5 µmol/L	0.473	ND	77
Additional Information: ND: Not determined due to the standard deviation cannot be calculated from n=2 replicates.					

CYP induction, Non-GLP			Test Article: Setmelanotide		
			Location in eCTD: 4.2.2.6-4		
			Study No.: [redacted]RHYTP2_CYP Induction		
Measured Concentrations of RM-493 in Culture Media on Day 3 of Treatment of Human Hepatocytes (Donor 1)					
Nominal Concentration (µmol/L)	**Parameter (n=1)**	**0 hr (dosing solution)**	**4 hr (post-dose)**	**8 hr (post-dose)**	**24 hr (post-dose)**
0.05	Measured Concentration (µmol/L)	0.0399	0.0304	0.0368	0.0352
	% of Nominal	80	61	74	70
	% of 0 hr	100	76	92	88
0.2	Measured Concentration (µmol/L)	0.260	0.252	0.425	0.382
	% of Nominal	130	126	213	191
	% of 0 hr	100	97	163	147
0.5	Measured Concentration (µmol/L)	0.493	0.471	0.709[a]	0.748[a]
	% of Nominal	99	94	142	150
	% of 0 hr	100	96	144	152
Measured Concentrations of RM-493 in Culture Media on Day 3 of Treatment of Human Hepatocytes (Donor 2)					
Nominal Concentration (µmol/L)	**Parameter (n=1)**	**0 hr (dosing solution)**	**4 hr (post-dose)**	**8 hr (post-dose)**	**24 hr (post-dose)**
0.05	Measured Concentration (µmol/L)	0.0626	0.0472	0.0491	0.0523
	% of Nominal	125	94	98	105
	% of 0 hr	100	75	78	84
0.2	Measured Concentration (µmol/L)	0.375	0.215	0.243	0.261
	% of Nominal	188	108	122	131
	% of 0 hr	100	57	65	70
0.5	Measured Concentration (µmol/L)	0.517	0.442	0.446	0.474
	% of Nominal	103	88	89	95
	% of 0 hr	100	85	86	92

a) The concentrations of RM-493 measured at 8 hr and 24 hr are higher than those at the early time points and the nominal. The reasons are unclear, possibly due to a high variability from sample preparation and injection.

CYP induction, Non-GLP		Test Article: Setmelanotide			
		Location in eCTD: 4.2.2.6-4			
		Study No.: [redacted]RHYTP2_CYP Induction			
Measured Concentrations of RM-493 in Culture Media on Day 3 of Treatment of Human Hepatocytes (Donor 3)					
Nominal Concentration (µmol/L)	**Parameter (n=1)**	**0 hr (dosing solution)**	**4 hr (post-dose)**	**8 hr (post-dose)**	**24 hr (post-dose)**
0.05	Measured Concentration (µmol/L)	0.0513	0.0587	0.0576	0.0570
	% of Nominal	103	117	115	114
	% of 0 hr	82	94	92	91
0.2	Measured Concentration (µmol/L)	0.238	0.271	0.296	0.352
	% of Nominal	119	136	148	176
	% of 0 hr	63	72	79	94
0.5	Measured Concentration (µmol/L)	0.499	0.512	0.604	0.629
	% of Nominal	100	102	121	126
	% of 0 hr	97	99	117	122
Additional Information: RM-493 had no cytotoxicity up to 1 µmol/L. RM-493 did not increase either mRNA or activity of CYP1A2, CYP2B6, or CYP3A at any of the three tested concentrations (0.05, 0.2, and 0.5 µmol/L) in any of the three tested human hepatocyte donors (<2-fold vs. vehicle control and <20% of positive control). Measurement of the concentrations of RM-493 in hepatocyte culture media on Day 3 of treatment of human hepatocytes indicated that RM-493 was stable in human hepatocytes during the induction experiment. In conclusion, RM-493 is unlikely to be an inducer of CYP1A2, CYP2B6, or CYP3A.					

Abbreviations: CYP = cytochrome P450; eCTD = electronic Common Technical Document; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; mRNA = messenger ribonucleic acid; OME = omeprazole; PB = phenobarbital; QC = quality control; qPCR = quantitative polymerase chain reaction; RIF = rifampicin; RM-493 = setmelanotide; SD = standard deviation; TA = test article.

2.6.5.13.5 膜透過性試験（791312）

Permeability Study, Non-GLP	**Test Article:** Setmelanotide
	Location in eCTD: 4.2.2.6-5
	Study No.: 791312
Type of Study: In Vitro	
Method: TC7 human intestinal epithelial cells (a subclone of Caco-2) were utilized to assess the apparent permeability (P_{app}) of the test and reference compounds, in the Apical (pH 6.5) to Basolateral (pH 7.4) and BL-AP permeability assays. RM-493 permeability was tested at 10 µmol/L. For AP-BL permeability, the donor sample times were 0 and 60 minutes, and receiver sample time was 60 minutes. For BL-AP permeability, the donor sample times were 0 and 40 minutes, and receiver sample time was 40 minutes. Quantitation was done by LC-MS/MS.	
Additional Information: For both AP-BL and BL-AP permeability assessments, RM-493 was detected in the donor samples, but was BLQ in the receiver samples. Thus, P_{app} could not be evaluated in this experiment.	

Abbreviations: AP-BL = apical-to-basolateral; BL-AP = basolateral-to-apical; BLQ = below the limit of quantitation; eCTD = electronic Common Technical Document; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; RM-493 = setmelanotide.

2.6.5.13.6 トランスポーターの基質認識性及び阻害試験（[redacted]RHYTP1）

Transporter, Non-GLP	**Test Article:** Setmelanotide
	Location in eCTD: 4.2.2.6-6
Type of Study: In Vitro	**Study No.:** [redacted]RHYTP1

Study System: The objective was to evaluate the substrate and inhibitor potential of setmelanotide for the following transporters: P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), organic anion transporting polypeptides 1B1 and 1B3 (OATP1B1 and OATP1B3), organic anion transporters 1 and 3 (OAT1 and OAT3), and organic cation transporter 2 (OCT2). See individual tables for each study design.

Design of Inhibitor Assessment Studies: HEK (human embryonic kidney 293) cell lines transfected with each of the uptake transporters of interest (OATP1B1, OATP1B3, OAT1, OAT3, or OCT2) were used to determine the inhibition potential of the test article. The uptake of a transporter-specific probe substrate was studied with and without the test article in transporter- and vector-transfected control cells. The test concentrations of the test article were as follows: for OATP1B1 and OATP1B3, 0.56 µmol/L; for OAT1, OAT3, and OCT2, 1.12 µmol/L. The cell media was gently aspirated, and the inhibition assay was initiated as follows: 1) incubation at 37°C with 5% CO_2 with 500 µL of probe substrate in the absence and presence of the test article or known inhibitor for the desired time; 2) at the end of the incubation period, the solution was gently aspirated; the cells were rinsed twice with ice-cold HBSSg 7.4 buffer; 3) the cells were lysed in 400 µL acetonitrile : water (3:1, v/v) containing IS; and 4) the lysates were collected for analysis of the probe substrate concentration by LC-MS/MS. The dosing solution concentrations of the test article in the inhibitor assessment were also determined by LC-MS/MS.

Net Influx Rate of Atorvastatin and Percent Inhibition: OATP1B1 and OATP1B3

Treatment	OATP1B1		OATP1B3	
	Net Influx Rate (pmol/mg protein/minute)	Percent Inhibition (%)	Net Influx Rate (pmol/mg protein/minute)	Percent Inhibition (%)
0.15 µmol/L Atorvastatin	7.09	NA	5.58	NA
0.15 µmol/L Atorvastatin + 0.56 µmol/L Setmelanotide	6.98	1.65	5.70	-2.10 (0[a])
0.15 µmol/L Atorvastatin + 10 µmol/L RSV	0.586	91.7	0.251	95.5

Transporter, Non-GLP	Test Article: Setmelanotide	
	Location in eCTD: 4.2.2.6-6	
Type of Study: In Vitro	Study No.: [redacted]RHYTP1	
Net Influx Rate of PAH and Percent Inhibition: OAT1		
Treatment	OAT1	
	Net Influx Rate (pmol/mg protein/minute)	Percent Inhibition (%)
10 µmol/L PAH	85.3	NA
10 µmol/L PAH + 1.12 µmol/L Setmelanotide	87.2	-2.19 (0[a])
10 µmol/L PAH + 100 µmol/L Probenecid	3.72	95.6
Net Influx Rate of Furosemide and Percent Inhibition: OAT3		
Treatment	OAT3	
	Net Influx Rate (pmol/mg protein/minute)	Percent Inhibition (%)
5 µmol/L furosemide	83.9	NA
5 µmol/L furosemide + 1.12 µmol/L Setmelanotide	86.3	-2.78 (0[a])
5 µmol/L furosemide + 100 µmol/L Probenecid	6.85	91.8
Net Influx Rate of MPP^+ and Percent Inhibition: OCT2		
Treatment	OCT2	
	Net Influx Rate (pmol/mg protein/minute)	Percent Inhibition (%)
5 µmol/L MPP^+	610	NA
5 µmol/L MPP^+ + 1.12 µmol/L Setmelanotide	626	-2.62 (0[a])
5 µmol/L MPP^+ + 300 µmol/L Imipramine	22.1	96.4

Additional Information:
a) The negative percentage inhibition indicates no (zero) inhibition.

Transporter, Non-GLP	**Test Article:** Setmelanotide
	Location in eCTD: 4.2.2.6-6
	Study No.: [redacted]RHYTP1

Design of Uptake Transporter Substrate Assessment: HEK cell lines transfected with each of the uptake transporters of interest (OATP1B1, OATP1B3, OAT1, OAT3, or OCT2) and vector control cells were used to determine the substrate potential of setmelanotide in a cellular uptake assay. The cells were incubated with different concentrations of the test article for 2, 5, and 10 min; the test concentrations were 0.01, 0.1, and 1 µmol/L in HBSSg 7.4 buffer. The cell media was gently aspirated, and the substrate assessments were initiated as follows: 1) incubation at 37°C with 5% CO_2 with 500 µL of test or control article solution for different periods of time; 2) at the end of the incubation period, the solution was gently aspirated; the cells were rinsed twice with ice-cold HBSSg 7.4 buffer (1,000 µL per rinse); 3) the cells were lysed in 400 µL acetonitrile : water (3:1, v/v) containing IS; and 4) the lysates (300 µL) were collected for analysis of test article or probe substrate concentrations. The assay was conducted stepwise: in the first step, a time course was run, and in the second step, a confirmatory step was run.

Uptake of Setmelanotide in VC, OATP1B1-, and OATP1B3-HEK Cells

Setmelanotide Concentration	Incubation (minutes)	2			5			10		
	Cell Line[a]	VC	1B1	1B3	VC	1B1	1B3	VC	1B1	1B3
		Influx Rate (pmol/minute/mg protein)								
0.01 µmol/L[b]	Replicate 1	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Replicate 2	0.000334	ND	ND	ND	ND	ND	ND	ND	ND
	Replicate 3	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Average	NA	NA	NA	NA	NA	NA	NA	NA	NA
	SD	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Influx Rate Ratio	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Influx Rate (pmol/minute/mg protein)								
0.1 µmol/L	Replicate 1	0.426	0.483	0.404	0.297	0.246	0.197	0.0956	0.0609	0.138
	Replicate 2	0.388	0.546	0.654	0.204	0.185	0.201	0.0819	0.0815	0.0977
	Replicate 3	0.737	0.531	0.532	0.352	0.112	0.182	0.179	0.0542	0.0992
	Average	0.517	0.520	0.530	0.284	0.181	0.194	0.119	0.0655	0.112
	SD	0.191	0.033	0.125	0.075	0.067	0.010	0.052	0.0142	0.023
	Influx Rate Ratio	NA	1.01	1.02	NA	0.637	0.681	NA	0.552	0.941
		Influx Rate (pmol/minute/mg protein)								
1 µmol/L	Replicate 1	7.80	6.27	3.28	2.25	2.74	1.36	1.17	0.865	1.03
	Replicate 2	8.15	6.05	2.96	2.30	2.83	1.23	1.33	0.707	0.964
	Replicate 3	8.76	5.43	3.09	2.68	1.56	1.34	1.16	0.856	0.580
	Average	8.24	5.91	3.11	2.41	2.38	1.31	1.22	0.809	0.858
	SD	0.49	0.44	0.16	0.24	0.71	0.07	0.10	0.089	0.243
	Influx Rate Ratio	NA	0.718	0.378	NA	0.987	0.543	NA	0.662	0.702

Additional Information: If setmelanotide had been a substrate of OATP1B1 or OATP1B3, the concentrations of the test article in the transporter-transfected cell lysates would be higher than those in the vector control-transfected cells.

a) VC: vector-transfected control cells; 1B1: OATP1B1-transfected cells; 1B3: OATP1B3-transfected cells.

b) The concentration of setmelanotide was less than the LLOQ (0.0001 µmol/L), and thus, the influx rate ratio could not be determined.

Transporter, Non-GLP	Test Article: Setmelanotide
	Location in eCTD: 4.2.2.6-6
	Study No.: [redacted]RHYTP1

Design of Uptake Transporter Substrate Assessment: HEK cell lines transfected with each of the uptake transporters of interest (OATP1B1, OATP1B3, OAT1, OAT3, or OCT2) and vector control cells were used to determine the substrate potential of setmelanotide in a cellular uptake assay. The cells were incubated with different concentrations of the test article for 2, 5, and 10 min; the test concentrations were 0.01, 0.1, and 1 µmol/L in HBSSg 7.4 buffer. The cell media was gently aspirated, and the substrate assessments were initiated as follows: 1) incubation at 37°C with 5% CO_2 with 500 µL of test or control article solution for different periods of time; 2) at the end of the incubation period, the solution was gently aspirated; the cells were rinsed twice with ice-cold HBSSg 7.4 buffer (1,000 µL per rinse); 3) the cells were lysed in 400 µL acetonitrile:water (3:1, v/v) containing IS; and 4) the lysates (300 µL) were collected for analysis of test article or probe substrate concentrations. The assay was conducted stepwise: in the first step, a time course was run, and in the second step, a confirmatory step was run.

Uptake of Setmelanotide in VC, OAT1-, and OAT3-HEK Cells

Setmelanotide Concentration	Incubation (minutes)	2			5			10		
	Cell Line[a]	VC	OAT1	OAT3	VC	OAT1	OAT3	VC	OAT1	OAT3
		Influx Rate (pmol/minute/mg protein)								
0.01 µmol/L[b]	Replicate 1	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Replicate 2	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Replicate 3	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Average	NA	NA	NA	NA	NA	NA	NA	NA	NA
	SD	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Influx Rate Ratio	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Influx Rate (pmol/minute/mg protein)								
0.1 µmol/L	Replicate 1	0.344	0.533	0.362	0.0873	0.193	0.285	0.0896	0.0898	0.123
	Replicate 2	0.234	0.391	0.528	0.171	0.137	0.389	0.0518	0.100	0.0971
	Replicate 3	0.232	0.303	0.289	0.131	0.122	0.254	0.108	0.105	0.0703
	Average	0.270	0.409	0.393	0.130	0.151	0.309	0.0832	0.0982	0.0969
	SD	0.064	0.116	0.123	0.042	0.037	0.071	0.0287	0.0076	0.0264
	Influx Rate Ratio	NA	1.52	1.46	NA	1.16	2.38	NA	1.18	1.16
		Influx Rate (pmol/minute/mg protein)								
1 µmol/L	Replicate 1	2.69	4.32	5.25	1.29	2.17	2.27	0.900	1.21	0.889
	Replicate 2	2.52	4.61	5.66	1.94	2.02	3.06	1.03	1.03	0.676
	Replicate 3	3.48	4.99	4.64	2.20	1.68	2.40	1.23	1.03	0.873
	Average	2.90	4.64	5.18	1.81	1.96	2.58	1.05	1.09	0.813
	SD	0.52	0.34	0.52	0.46	0.25	0.42	0.17	0.11	0.118
	Influx Rate Ratio	NA	1.60	1.79	NA	1.08	1.42	NA	1.04	0.771

Additional Information: If setmelanotide had been a substrate of OAT1 or OAT3, the concentrations of the test article in the transporter-transfected cell lysates would be higher than those in the vector control-transfected cells. A confirmatory step was run for OAT3 and setmelanotide was found to not be a substrate of OAT3.

a) VC: vector-transfected control cells; OAT1: OAT1-transfected cells; OAT3: OAT3-transfected cells.

b) The concentration of setmelanotide was less than the LLOQ (0.0001 µmol/L), and thus, the influx rate could not be determined.

Transporter, Non-GLP		Test Article: Setmelanotide					
		Location in eCTD: 4.2.2.6-6					
		Study No.: [redacted]RHYTP1					
Uptake of Setmelanotide in VC- and OCT2-HEK Cells							
Setmelanotide Concentration	Incubation (minutes)	2		5		10	
	Cell Line[a]	VC	OCT2	VC	OCT2	VC	OCT2
		Influx Rate (pmol/minute/mg protein)					
0.01 µmol/L[b]	Replicate 1	ND	ND	ND	ND	ND	ND
	Replicate 2	ND	ND	ND	ND	ND	ND
	Replicate 3	ND	ND	ND	ND	ND	ND
	Average	NA	NA	NA	NA	NA	NA
	SD	NA	NA	NA	NA	NA	NA
	Influx Rate Ratio	NA	NA	NA	NA	NA	NA
		Influx Rate (pmol/minute/mg protein)					
0.1 µmol/L	Replicate 1	1.38	1.43	0.723	0.464	0.263	0.220
	Replicate 2	1.63	1.19	0.698	0.406	0.176	0.134
	Replicate 3	1.56	1.32	0.725	0.429	0.309	0.274
	Average	1.52	1.32	0.715	0.433	0.249	0.209
	SD	0.13	0.12	0.015	0.029	0.068	0.071
	Influx Rate Ratio	NA	0.864	NA	0.605	NA	0.839
		Influx Rate (pmol/minute/mg protein)					
1 µmol/L	Replicate 1	12.7	14.8	7.47	4.53	2.73	1.85
	Replicate 2	13.0	14.4	7.40	3.85	4.17	2.17
	Replicate 3	14.4	14.9	5.44	3.19	2.31	2.53
	Average	13.4	14.7	6.77	3.86	3.07	2.19
	SD	0.9	0.3	1.15	0.67	0.98	0.34
	Influx Rate Ratio	NA	1.10	NA	0.570	NA	0.712

Additional Information: If setmelanotide had been a substrate of OCT2, the concentrations of the test article in the transporter-transfected cell lysates would be higher than those in the vector control-transfected cells.

a) VC: vector-transfected control cells; OCT2: OCT2-transfected cells.

b) The concentration of setmelanotide was less than the LLOQ (0.0001 µmol/L); the influx rate could not be determined.

Transporter, Non-GLP	Test Article: Setmelanotide	
	Location in eCTD: 4.2.2.6-6	
	Study No.: [redacted]RHYTP1	
Efflux Ratio of Digoxin and Percent Inhibition – P-gp Inhibitor Assessment		
Treatment	**Corrected Efflux Ratio**	**Percent Inhibition**
10 µmol/L Digoxin	204	NA
10 µmol/L Digoxin + 1.12 µmol/L Setmelanotide	271	-33.0 (0[a])
10 µmol/L Digoxin + 1 µmol/L Valspodar	0.655	99.7
Additional Information: a) The negative percent inhibition indicates no (zero) inhibition.		
Efflux Ratio of Cladribine and Percent Inhibition – BCRP Inhibitor Assessment		
Treatment	**Corrected Efflux Ratio**	**Percent Inhibition**
10 µmol/L Cladribine	97.2	NA
10 µmol/L Cladribine + 1.12 µmol/L Setmelanotide	74.3	23.6
10 µmol/L Cladribine + 0.5 µmol/L Ko143	0.0586	99.9
Study Design for P-gp and BCRP Substrate Assessment: The P-gp and BCRP substrate assessment of setmelanotide was conducted in MDR1-MDCK, BCRP-MDCK, and MDCK cells. LY was co-dosed with each dosing solution and the nominal concentration of LY was 200 µmol/L. Aliquots of fresh dosing solution (0.55 mL for the AP-to-BL, 1.55 mL for the BL-to-AP) were added to the donor compartment, and HBSSg 7.4 buffer (1.5 mL for the AP-to-BL, 0.5 mL for the BL-to-AP) were added to the receiver compartment. For receiver samples, aliquots (300 µL) were taken at the pre-selected time points, and replaced with equal volume of fresh HBSSg 7.4 buffer, except for the last time point. For the receiver samples, one part (200 µL) was used for the analysis of setmelanotide, and the rest of the sample (100 µL) was collected for the LY measurement at the end of permeability assay. For donor samples, aliquots (50 µL) were taken at the selected time points without replacement. In addition to the above treatments, the bidirectional permeability of setmelanotide was also assessed in the presence of 1 µmol/L valspodar (a P-gp inhibitor) in MDR1-MDCK cells or 0.5 µmol/L Ko143 (a BCRP inhibitor) in BCRP-MDCK cells. A 30-minute pre-incubation with the known inhibitor solution was performed to pre-load the cells with inhibitor; the pre-incubation solution was placed in both AP and BL sides of cell monolayers. After 30 minutes, the pre-incubation solution was aspirated, and the permeability assay was initiated as described above. In addition to the test article treatment, the transport of digoxin (MDR1-MDCK) and cladribine (BCRP-MDCK) was also measured in the absence and presence of valspodar or Ko143, respectively. The concentrations of setmelanotide collected from the receiver and donor sides were analyzed with LC-MS/MS. The concentrations of LY were determined by fluorescence using a BMG microplate reader with excitation and emission wavelengths at 485 nm and 540 nm, respectively.		

Transporter, Non-GLP	Test Article: Setmelanotide
	Location in eCTD: 4.2.2.6-6
	Study No.: [redacted]RHYTP1

Permeability and Recovery of Setmelanotide in MDCK Cells

Treatment	Replicates	AP-to-BL		BL-to-AP		Efflux Ratio
		P_{app} (10^{-6}cm/s)	Recovery (%)	P_{app} (10^{-6}cm/s)	Recovery (%)	
0.01 µmol/L Setmelanotide	1	ND[a]	<48.4[a]	2.77[b]	46.4[c]	NA
	2	ND[a]	<51.2[a]	ND[a]	<30.4[a]	
	3	ND[a]	<54.3[a]	ND[a]	<27.4[a]	
	Average	NA	NA	NA	NA	
	SD	NA	NA	NA	NA	
0.1 µmol/L Setmelanotide	1	ND[a]	<81.2[a]	ND[a]	<72.2[a]	NA
	2	ND[a]	<78.0[a]	ND[a]	<72.2[a]	
	3	ND[a]	<79.1[a]	ND[a]	<72.5[a]	
	Average	NA	NA	NA	NA	
	SD	NA	NA	NA	NA	
1 µmol/L Setmelanotide	1	3.10[b]	90.6	0.0107[d]	102	0.00619
	2	2.81[b]	97.3	0.0248	102	
	3	2.66[b]	102	0.0175[b]	96.9[c]	
	Average	2.86	96.6	0.0177	100	
	SD	0.22	5.7	0.0071	2.9	

Additional Information:

a) The receiver concentration of setmelanotide was less than the LLOQ (0.0001 µmol/L); the P_{app} value was not determined.

b) The P_{app} was determined with Equation $P_{app} = \frac{C_R^{final}/60 \times V_R}{A \times C_{D0} \times 60}$: where A = cell membrane surface area; C_{D0} = 0-minute donor concentration; C_R^{final} = cumulative receiver concentration at the end of the incubation; P_{app} = apparent permeability; V_R = volume of the receiver compartment.

c) The recovery was determined with 60-minute receiver and donor concentrations.

d) The P_{app} was determined with Equation $P_{app} = \frac{C_R^{final}/120 \times V_R}{A \times C_{D0} \times 60}$: where A = cell membrane surface area; C_{D0} = 0-minute donor concentration; C_R^{final} = cumulative receiver concentration at the end of the incubation; P_{app} = apparent permeability; V_R = volume of the receiver compartment.

Transporter, Non-GLP	Test Article: Setmelanotide
	Location in eCTD: 4.2.2.6-6
	Study No.: [redacted]RHYTP1

Permeability and Recovery of Setmelanotide in MDR1-MDCK Cells

Treatment	Replicates	AP-to-BL		BL-to-AP		Efflux Ratio
		P_{app} (10^{-6}cm/s)	Recovery (%)	P_{app} (10^{-6}cm/s)	Recovery (%)	
0.01 µmol/L Setmelanotide	1	ND[a]	<35.4[a]	ND[a]	15.4[a]	NA
	2	ND[a]	35.3[a]	ND[a]	14.7[a]	
	3	ND[a]	38.9[a]	ND[a]	19.4[a]	
	Average	NA	NA	NA	NA	
	SD	NA	NA	NA	NA	
0.1 µmol/L Setmelanotide	1	ND[a]	68.1[a]	ND[a]	60.7[a]	NA
	2	ND[a]	72.3[a]	ND[a]	57.3[a]	
	3	ND[a]	73.9[a]	ND[a]	64.8[a]	
	Average	NA	NA	NA	NA	
	SD	NA	NA	NA	NA	
1 µmol/L Setmelanotide	1	7.24[b]	94.6	0.0831	89.0	0.0222
	2	3.92[b]	95.5	0.0996	95.3	
	3	0.110	93.7	0.0678	95.4	
	Average	3.75	94.6	0.0835	93.3	
	SD	3.57	0.9	0.0159	3.7	
0.1 µmol/L Setmelanotide +1 µmol/L Valspodar	1	ND[a]	63.9	ND[a]	63.6	NA
	2	ND[a]	72.1	ND[a]	58.9	
	3	0.438	71.1	ND[a]	60.0	
	Average	NA	NA	NA	NA	
	SD	NA	NA	NA	NA	

Additional Information:

a) The receiver concentration of setmelanotide was less than the LLOQ (0.0001 µmol/L); the P_{app} value was not determined.

b) The P_{app} was determined with Equation $P_{app} = \frac{C_R^{final}/60 \times V_R}{A \times C_{D0} \times 60}$: where A = cell membrane surface area; C_{D0} = 0-minute donor concentration; C_R^{final} = cumulative receiver concentration at the end of the incubation; P_{app} = apparent permeability; V_R = volume of the receiver compartment.

Transporter, Non-GLP	Test Article: Setmelanotide
	Location in eCTD: 4.2.2.6-6
	Study No.: [redacted]RHYTP1

Permeability and Recovery of Setmelanotide in BCRP-MDCK Cells – BCRP Substrate Assessment

Treatment	Replicates	AP-to-BL		BL-to-AP		Efflux Ratio
		P_{app} (10^{-6}cm/s)	Recovery (%)	P_{app} (10^{-6}cm/s)	Recovery (%)	
0.01 µmol/L Setmelanotide	1	ND[a]	<58.0[a]	ND[a]	<28.9[a]	NA
	2	ND[a]	<55.3[a]	ND[a]	<20.9[a]	
	3	ND[a]	<63.6[a]	ND[a]	<39.5[a]	
	Average	NA	NA	NA	NA	
	SD	NA	NA	NA	NA	
0.1 µmol/L Setmelanotide	1	ND[a]	<105[a]	ND[a]	<101[a]	NA
	2	3.15	118	ND[a]	<110[a]	
	3	1.36	103	ND[a]	<106[a]	
	Average	2.26	110	NA	NA	
	SD	ND	ND	NA	NA	
1 µmol/L Setmelanotide	1	0.0484	76.0	0.0371	81.6	0.525
	2	0.0576	69.2	0.0213	82.8	
	3	0.386[b,c]	64.8	0.0250	74.8	
	Average	0.0530	70.0	0.0278	79.7	
	SD	ND	5.6	0.0082	4.3	
0.1 µmol/L Setmelanotide + 0.5 µmol/L Ko143	1	ND[a]	<68.6[a]	ND[a]	<64.3[a]	NA
	2	ND[a]	<65.6[a]	ND[a]	<64.5[a]	
	3	ND[a]	<69.1[a]	ND[a]	<72.5[a]	
	Average	NA	NA	NA	NA	
	SD	NA	NA	NA	NA	

Additional Information:

a) The receiver concentration of setmelanotide was less than the LLOQ (0.0001 µmol/L); the P_{app} value was not determined.

b) The P_{app} was determined with Equation $P_{app} = \frac{C_R^{final}/60 \times V_R}{A \times C_{D0} \times 60}$: where A = cell membrane surface area; C_{D0} = 0-minute donor concentration; C_R^{final} = cumulative receiver concentration at the end of the incubation; P_{app} = apparent permeability; V_R = volume of the receiver compartment.

c) The P_{app} value was a statistical outlier and was excluded from the calculation of the average.

Overall Conclusions: RM-493 is very unlikely to be a substrate of P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, or OCT2. RM-493 is not a significant inhibitor of P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, or OCT2 at the tested, clinically relevant concentrations.

Abbreviations: AP = apical; BL = basolateral; BCRP = breast cancer resistance protein; eCTD = electronic Common Technical Document; GLP = Good Laboratory Practice; HBSSg = Hank's balanced salt solution containing 10 mmol/L N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid and 15 mmol/L D-glucose; HEK = human embryonic kidney; IS = internal standard; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantitation; LY = lucifer yellow; MDCK = Madin-Darby canine kidney cells; MDR1 = multidrug resistance protein 1; MPP^+ = 1-methyl-4-phenylpyridinium iodide; NA = not appliable; ND = not determined; OAT = organic anion transporter; OATP = organic anion transporting polypeptide; OCT = organic cation transporter; P-gp = P-glycoprotein; PAH = para-aminohippurate; P_{app} = apparent permeability; RM-493 = setmelanotide; RSV = rosuvastatin; SD = standard deviation; VC = vector-transfected control.

2.6.5.13.7 MATE1 及び MATE2K の基質認識性及び阻害試験（[redacted]RHYTP3）

Transporter, Non-GLP					**Test Article:** Setmelanotide					
					Location in eCTD: 4.2.2.6-7					
					Study No.: [redacted]RHYTP3					
Study System: In vitro – Human embryonic kidney (HEK293) epithelial cells transfected with each of efflux transporters (MATE1 or MATE2K) were used to determine the substrate potential of RM-493 in a cellular uptake assay. HEK293 cells transfected with an individual efflux transporter were run in parallel with vector control cells. The cells were incubated with RM-493 (2.1 µmol/L) for 2, 5 and 10 minutes in HBSSg buffer, pH 8.5.										
Substrate Assessment: Influx Rate and Influx Rate Ratios of RM-493 in MATE1-, MATE2K-, and Vector-transfected HEK Cells										
Concentrations of RM-493 (µmol/L)	**Incubation (min)**	**2**			**5**			**10**		
	Cell Line	VC	MATE1	MATE2K	VC	MATE1	MATE2K	VC	MATE1	MATE2K
	Replicate	**Influx Rate (pmol/minute/mg protein)**								
2.1 µmol/L RM-493	**1**	6.01	11.8	15.7	3.41	7.58	6.01	1.58	1.94	1.56
	2	8.02	8.30	12.5	4.16	5.33	6.61	1.69	2.26	2.22
	3	6.35	9.21	16.0	5.88	6.11	6.65	1.28	2.19	2.54
	Mean	6.79	9.78	14.7	4.48	6.34	6.42	1.52	2.13	2.11
	SD	1.07	1.83	1.95	1.26	1.14	0.361	0.216	0.170	0.497
	Ratio (IRR)	*NA*	*1.44*	*2.17*	*NA*	*1.41*	*1.43*	*NA*	*1.41*	*1.39*

Transporter, Non-GLP	Test Article: Setmelanotide
	Location in eCTD: 4.2.2.6-7
	Study No.: [redacted]RHYTP3

Influx Rate of Metformin in the Absence and Presence of RM-493 or Cimetidine in MATE1- and Vector-transfected HEK293 Cells (MATE1 Inhibitor Assessment)

Treatment	Replicate	Influx Rate (pmol/min/mg protein)	
		VC(G418)-HEK293	MATE1-HEK293
50 µmol/L Metformin	1	15.9	246
	2	15.2	217
	3	17.9	230
	Average	16.3	231
	SD	1.40	14.5
	Carrier-mediated	NA	214
50 µmol/L Metformin + 1.1 µmol/L RM-493	1	23.3	229
	2	33.5	218
	3	15.0	223
	Average	23.9	224
	SD	9.27	5.63
	Carrier-mediated	NA	200
	Percentage Inhibition	NA	6.83
50 µmol/L Metformin + 100 µmol/L Cimetidine	1	14.1	19.4
	2	26.0	31.4
	3	20.6	25.9
	Average	20.2	25.6
	SD	5.95	6.01
	Carrier-mediated	NA	5.39
	Percentage Inhibition	NA	97.5

Transporter, Non-GLP	Test Article: Setmelanotide
	Location in eCTD: 4.2.2.6-7
	Study No.: [redacted]RHYTP3

Influx Rate of Metformin in the Absence and Presence of RM-493 or Pyrimethamine in MATE2K- and Vector-transfected HEK293 Cells (MATE2K Inhibitor Assessment)

Treatment	Replicate	Influx Rate (pmol/min/mg protein)	
		VC(G418)-HEK293	MATE2K-HEK293
50 µmol/L Metformin	1	15.9	51.4
	2	15.2	50.6
	3	17.9	52.8
	Average	16.3	51.6
	SD	1.40	1.12
	Carrier-mediated	NA	35.3
50 µmol/L Metformin + 1.1 µmol/L RM-493	1	23.3	55.2
	2	33.5	47.4
	3	15.0	61.1
	Average	23.9	54.5
	SD	9.27	6.88
	Carrier-mediated	NA	30.6
	Percentage Inhibition	NA	13.1
50 µmol/L Metformin + 10 µmol/L Pyrimethamine	1	20.8	18.8
	2	17.0	22.0
	3	19.2	14.8
	Average	19.0	18.5
	SD	1.92	3.62
	Carrier-mediated	NA	-0.458
	Percentage Inhibition	NA	100[a]

Additional Information: Under the test conditions: 1) RM-493 is a non-substrate of MATE1 and a borderline substrate of MATE2K; 2) RM-493 is not an inhibitor of MATE1 or MATE2K. The MATE1 and MATE2K influx rate ratios of metformin (a probe substrate of MATE1 and MATE2K) were 14.1 (MATE1-over vector-transfected HEK293 cells), and 3.16 (MATE2K over vector-transfected HEK293 cells), indicating the normal function of the test systems.

a) The percentage inhibition was greater than 100; the value of 100 was reported.

Abbreviations: eCTD = electronic Common Technical Document; GLP = Good Laboratory Practice; HBSSg = Hank's balanced salt solution containing 10 mmol/L N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid and 15 mmol/L D-glucose; HEK = human embryonic kidney; IRR = influx rate ratio; MATE = multidrug and toxin extrusion; NA = not applicable; RM-493 = setmelanotide; SD = standard deviation; VC = vector-transfected control.

補足薬物動態/ADME 試験

2.6.5.14 薬物動態：ラットにおける単回投与時の吸収試験（予備試験）

2.6.5.14.1 生理食塩液による単回皮下投与薬物動態試験（RM-493-PK-005）

Rat PK, Non-GLP	**Test Article:** Setmelanotide			
	Location in eCTD: 4.2.2.7-1			
	Study No: RM-493-PK-005			
Species/Strain:	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**	**Rat/ Sprague-Dawley**
Gender (M/F)/Number of animals:	24M	24M	24M	24M
Feeding condition:	Fed	Fed	Fed	Fed
Vehicle/Formulation:	RM-493 in Saline	RM-493 in Saline	RM-493 in Saline	RM-493 in Saline
Method of Administration:	SC Injection	SC Injection	SC Injection	SC Injection
Dose (mg/kg):	3	10	30	90
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma	Plasma
Analyte:	RM-493	RM-493	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters:				
t_{max} (hr)	0.50	1.00	1.00	0.50
C_{max} (ng/mL)	741	1,280	2,030	2,850
C_{max}/Dose ([ng/mL]/[mg/kg])	247	128	67.7	31.7
AUC_{last} (ng*hr/mL)	1,507	3,815	6,998	12,940
AUC_{∞} (ng*hr/mL)	1,515	3,824	7,009	12,960
AUC_{∞}/Dose ([ng*hr/mL]/[mg/kg])	505.1	382.4	233.6	144.0
AUC_{Extrap} (%)	0.51	0.23	0.16	0.13
$t_{1/2}$ (hr)	1.01	1.41	3.59	4.94
t_{last} (hr)	8.00	12.00	24.00	48.00
C_{last} (ng/mL)	5.30	4.31	2.15	2.40
CL/F (L/hr/kg)	1.980	2.615	4.280	6.946
V_z/F (L/kg)	2.887	5.334	22.18	49.52

Abbreviations: AUC = area under the plasma concentration-time curve; AUC_{∞} = AUC from time 0 to infinity; AUC_{∞}/Dose = dose-normalized AUC from time 0 to infinity; AUC_{Extrap} = the percentage of AUC_{∞} based on extrapolation; AUC_{last} = AUC from time 0 to the last measurable timepoint; C_{last} = last measurable plasma concentration; CL/F = apparent plasma clearance; C_{max} = maximum plasma concentration; C_{max}/Dose = dose-normalized C_{max}; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; PK = pharmacokinetic; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; t_{last} = time of last measurable plasma concentration; t_{max} = time to C_{max}; V_z/F = apparent volume of distribution.

2.6.5.15 薬物動態：サルにおける単回投与及び反復投与時の吸収試験（予備試験）

2.6.5.15.1 肥満アカゲザルにおける単回投与薬物動態試験（BIM-22493-018）

Monkey PK, Non-GLP	**Test Article:** Setmelanotide	
	Location in eCTD: 4.2.2.7-2	
	Study No.: BIM-22493-018	
Species/Strain:	**Monkey/Rhesus Macaque**	**Monkey/Rhesus Macaque**
Gender (M/F)/Number of animals:	3M/3F	3M/3F
Feeding condition:	Fed	Fed
Vehicle/Formulation:	Saline (2% heat inactivated monkey serum)	Saline (2% heat inactivated monkey serum)
Method of Administration:	SC Injection	SC Infusion
Dose:	450 nmol/kg (0.5 mg/kg)	450 nmol/kg (0.5 mg/kg)
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma
Analyte:	RM-493	RM-493
Assay:	LC-MS/MS	LC-MS/MS
Mean PK parameters:		
t_{max} (hr)	2	1.5[a]
C_{max} (ng/mL)	951	~150
$t_{1/2}$ (hr)	6.1	NR
AUC (ng*hr/mL)	10,870	3,813 (24 hr)

Additional Information:

a) Although the report table says that t_{max} was 1.5 hr, four animals had a t_{max} of 24 hr and two animals had a t_{max} of 2 hr.

Abbreviations: AUC = area under the plasma concentration-time curve; C_{max} = maximum plasma concentration; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; NR = not reported; PK = pharmacokinetic; RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; t_{max} = time to C_{max}.

2.6.5.15.2 肥満アカゲザルにおける各種製剤による反復投与薬物動態試験（RM-493-PK-002）

Monkey PK, Non-GLP	**Test Article:** Setmelanotide				
	Location in eCTD: 4.2.2.7-3				
	Study No.: RM-493-PK-002				
Species/Strain:	**Monkey/ Rhesus Macaque**	**Monkey/ Rhesus Macaque**	**Monkey/ Rhesus Macaque**	**Monkey/ Rhesus Macaque**	**Monkey/ Rhesus Macaque**
Gender (M/F)/Number of animals:	3M/3F	6M/6F	6M/6F	6M/6F	3M/3F
Feeding condition:	Fed	Fed	Fed	Fed	Fed
Vehicle/Formulation:	Acetate Salt in Saline (Experiment 2)	Acetate Salt in Saline (Experiment 5)	Sterile saline + 2% heat-inactivated serum (Experiment 6)	Sterile saline + 2% heat-inactivated serum (Experiment 6)	Microparticle BJ-36 in Miglyol and Lecithin (Experiment 4)
Method of Administration:	SC Injection	SC Injection	SC Infusion[a]	SC Infusion[a]	SC Injection
Dose (mg/kg):	0.19 BID x 5 days	0.19 /0.25 BID x 27 days	0.5 x 3 days (450 nmol/kg/day)	~0.5 x 3 days (450 nmol/kg/day)	0.5 QD x 4 days
Sample (e.g., whole blood, plasma, serum):	Plasma	Plasma	Plasma, CSF	Plasma, CSF	Plasma
Analyte:	RM-493	RM-493	RM-493	LY2112688	RM-493
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Mean PK parameters:	**Day 1**	**Day 27, Males only**	**Day 1 AM (24 hr)**	**Day 1 AM (24 hr)**	**Day 4**
t_{max} (hr)	1.00	0.85	NC	NC	6.67
C_{max} (ng/mL)	437	822	NC	NC	430
AUC_{last} (ng*hr/mL)	2,060	6,990	NC	NC	6,020
$t_{1/2}$ (hr)	3.51	5.61	NC	NC	3.29
C_{avg} (ng/mL)	NC	500	339	275	243

Monkey PK, Non-GLP	**Test Article:** Setmelanotide				
	Location in eCTD: 4.2.2.7-3				
	Study No.: RM-493-PK-002				
Species/Strain:	**Monkey/ Rhesus Macaque**	**Monkey/ Rhesus Macaque**	**Monkey/ Rhesus Macaque**	**Monkey/ Rhesus Macaque**	**Monkey/ Rhesus Macaque**
Vehicle/Formulation:	Acetate Salt in Saline (Experiment 2)	Acetate Salt in Saline (Experiment 5)	Sterile saline + 2% heat-inactivated serum (Experiment 6)	Sterile saline + 2% heat-inactivated serum (Experiment 6)	Microparticle BJ-36 in Miglyol and Lecithin (Experiment 4)
Method of Administration:	SC Injection	SC Injection	SC Infusion[a]	SC Infusion[a]	SC Injection
Dose (mg/kg):	0.19 BID x 5 days	0.19 /0.25 BID x 27 days	0.5 x 3 days (450 nmol/kg/day)	~0.5 x 3 days (450 nmol/kg/day)	0.5 QD x 4 days
	Day 5 AM		**Day 3 AM (72 hr)**	**Day 3 AM (72 hr)**	
t_{max} (hr)	1.29	NC	NC	NC	NC
C_{max} (ng/mL)	365	NC	NC	NC	NC
AUC_{last} (ng*hr/mL)	1,820	NC	NC	NC	NC
$t_{1/2}$ (hr)	3.28	NC	NC	NC	NC
C_{avg}[b] (ng/mL)	NC	NC	495	271	NC
CSF_{avg}[b] (ng/mL)	NC	NC	0.788	1.57	NC
	Day 5 PM				
t_{max} (hr)	1.38	NC	NC	NC	NC
C_{max} (ng/mL)	473	NC	NC	NC	NC
AUC_{last} (ng*hr/mL)	3,520	NC	NC	NC	NC
$t_{1/2}$ (hr)	3.85	NC	NC	NC	NC

Additional Information: Experiment 5 results exclude 1 animal due to concentrations 3-fold higher than other animals.

a) Continuous 24-hr/day infusion.

b) C_{avg} and CSF_{avg} collected at the same time, ~10 AM on Day 3.

Abbreviations: AUC_{last} = area under the plasma concentration-time curve from time 0 to the last measurable timepoint; BID = twice daily; C_{avg} = average plasma concentration; C_{max} = maximum plasma concentration; CSF = cerebral spinal fluid; CSF_{avg} = average cerebral spinal fluid concentration; eCTD = electronic Common Technical Document; F = female; GLP = Good Laboratory Practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; NC = not calculated; PK = pharmacokinetic; QD = quaque die (once daily); RM-493 = setmelanotide; SC = subcutaneous; $t_{1/2}$ = half-life; t_{max} = time to C_{max}.