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2.6.7.1 毒性試験：一覧表

Test Article: Setmelanotide (RM-493)							
Type of Study	**Species and Strain**	**Method of Administration**	**Duration of Dosing**	**Doses (mg/kg/day)[ab] (vehicle)**	**GLP**	**Testing Facility**	**Study Number / Location in eCTD**
Repeat-Dose Toxicity							
28-day dose range-finding toxicity	Sprague Dawley rat	Subcutaneous injection	28 days	0 (mPD), 2, 10, 20, 30 (in mPD), 30 (in saline)	No	[redacted] Canada	RM-493-TOX-026 / 4.2.3.2-1
28-day dose range-finding toxicity	Sprague Dawley rat	Subcutaneous injection	28 days	0 (mPD), 1, 3, 5 (in mPD), 2.5, 5, 7.5, 15, 30 (in saline)	No	[redacted] Canada	RM-493-TOX-036 / 4.2.3.2-2
26-week toxicity with 4-week recovery	Sprague Dawley rat	Subcutaneous injection	26 weeks	0 (saline), 0 (mPD), 3, 15 (in saline), 0.5, 1.5, 3 (in mPD)	Yes	[redacted] Canada	RM-493-TOX-023 / 4.2.3.2-3
28-day dose range-finding toxicity	Cynomolgus monkey	Subcutaneous injection	28 days	0 (mPD), 0.2, 0.75, 2 (in mPD); 2 (in saline)	No	[redacted] Canada	RM-493-TOX-027 / 4.2.3.2-4
39-week toxicity with 4-week recovery	Cynomolgus monkey	Subcutaneous injection	39 weeks	0 (saline), 0 (mPD), 0.5/0.25, 1.5/0.5, 3/1 (in mPD), 3 (in saline)	Yes	[redacted] Canada	RM-493-TOX-024 / 4.2.3.2-5
Genotoxicity							
Bacterial reverse mutation	*S. typhirurium* (TA98, TA100, TA1535, TA1537) *E. coli* (WP2*uvrA*)	In vitro	65 hours	Up to 5,000 µg/plate	Yes	[redacted] Canada	RM-493-TOX-008 / 4.2.3.3.1-1
Chromosome aberration	Human (peripheral blood lymphocytes)	In vitro	4 and 21 hours	Up to 5,000 µg/mL	Yes	[redacted] Canada	RM-493-TOX-009 / 4.2.3.3.1-2
Micronucleus	Rat	Continuous subcutaneous infusion	2 days	0, 50, 100, 200 (in saline)	Yes	[redacted] Canada	RM-493-TOX-020 / 4.2.3.3.2-1

Test Article: Setmelanotide (RM-493)							
Type of Study	**Species and Strain**	**Method of Administration**	**Duration of Dosing**	**Doses (mg/kg/day)[ab] (vehicle)**	**GLP**	**Testing Facility**	**Study Number / Location in eCTD**
Carcinogenicity							
Dose range-finding	rasH2 mouse, wild-type	Subcutaneous injection	7 days	0 (mPD), 10, 20, 40, 50, 60, 80, 100 (in mPD)	No	[redacted], Canada	RM-493-TOX-040 / 4.2.3.4.2-1
Dose range-finding	rasH2 mouse, wild-type	Subcutaneous injection	28 days	0 (saline), 0 (mPD), 10, 20 (in mPD)	Yes	[redacted], Canada	RM-493-TOX-041 / 4.2.3.4.2-2
26-week carcinogenicity	Tg.rasH2 mice	Subcutaneous injection	26 weeks	0 (saline), 0 (mPD), 1, 3, 10 (in mPD) 75 (positive control)	Yes	[redacted], Canada	RM-493-TOX-042 / 4.2.3.4.2-3
26-week carcinogenicity	Tg.rasH2 mice	Subcutaneous injection	26 weeks	0 (saline), 0 (mPD), 1, 3, 10 (in mPD) 75 (positive control)	Yes	[redacted] Canada	RM-493-TOX-044 / 4.2.3.4.2-4
26-week carcinogenicity	Tg.rasH2 mice	Subcutaneous injection	26 weeks	0 (saline), 0 (mPD), 1, 3, 10 (in mPD) 75 (positive control)	Yes	[redacted] Canada	RM-493-TOX-045 / 4.2.3.4.2-5
Reproductive and Developmental Toxicity							
Male fertility	Sprague Dawley rat	Subcutaneous injection	28 days prior to and during mating	0 (saline), 0 (mPD), 3, 15 (saline), 0.5, 1.5, 3 (in mPD)	Yes	[redacted], Canada	RM-493-TOX-023 / 4.2.3.2-3
Female fertility/ EFD	Sprague Dawley rat	Subcutaneous injection	14 days prior to mating to GD 17	0 (saline), 0 (mPD), 0.5, 3, 5 (in mPD)	Yes	[redacted], Canada	RM-493-TOX-029 / 4.2.3.5.1-1
Dose range-finding EFD	NZW rabbit	Subcutaneous injection	GD 7-13 GD 13-19	0 (mPD), 0.05, 0.5, 3, 10, 20 (in mPD)	No	[redacted], Canada	RM-493-TOX-030 / 4.2.3.5.2-1
EFD	NZW rabbit	Subcutaneous injection	GD 7-13 GD 13-19	0 (mPD), 0.05, 0.1, 0.2 (in mPD)	Yes	[redacted] Canada	RM-493-TOX-031 / 4.2.3.5.2-2
Pre/postnatal development	Sprague Dawley rat	Subcutaneous injection	GD 6 to LD 21	0 (saline), 0 (mPD), 0.5, 3, 5 (in mPD)	Yes	[redacted], Canada	RM-493-TOX-033 / 4.2.3.5.3-1

Test Article: Setmelanotide (RM-493)							
Type of Study	**Species and Strain**	**Method of Administration**	**Duration of Dosing**	**Doses (mg/kg/day)[ab] (vehicle)**	**GLP**	**Testing Facility**	**Study Number / Location in eCTD**
Juvenile Toxicity							
Juvenile dose range-finding	Sprague Dawley rat	Subcutaneous injection	28 days	0 (mPD), 1, 2.5, 5, (in mPD) 15 (in saline)	No	[redacted], Canada	RM-493-TOX-034 / 4.2.3.5.4-1
Juvenile toxicity	Sprague Dawley rat	Subcutaneous injection	7 weeks	0 (saline), 0 (mPD), 0.5, 1.5, 3 (in mPD), 15 (in saline)	Yes	[redacted], Canada	RM-493-TOX-035 / 4.2.3.5.4-2
Local Tolerance							
Subcutaneous Irritation	Sprague Dawley rat	Subcutaneous injection	7 days	0 (saline), 0 (mPD), 5, 10 (in mPD)	Yes	[redacted], US	RM-493-TOX-022 / 4.2.3.6-1
Other Toxicity							
13-week toxicity with 4-week recovery	Sprague Dawley rat	Continuous subcutaneous infusion	13 weeks	0 (saline), 10, 30, 60 (in saline)	Yes	[redacted] Canada	RM-493-TOX-016 / 4.2.3.7.7-1
13-week toxicity with 10-day recovery	Cynomolgus monkey	Continuous subcutaneous infusion	13 weeks	0 (saline), 1, 10, 25 (in saline)	Yes	[redacted], Canada	RM-493-TOX-015 / 4.2.3.7.7-2

EFD, embryo-fetal development; GD, gestation day; GLP, Good Laboratory Practice; LD, lactation day; mPD, mPEG-DSPE (N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine); NZW, New Zealand white.

a Unless indicated otherwise.

b Underlined doses indicate the no-observed adverse effect level (NOAEL).

2.6.7.2 トキシコキネティクス：トキシコキネティクス試験の一覧表

					Test Article: Setmelanotide (RM-493)
Type of Study	**Test System**	**Method of Administration**	**Doses (mg/kg)**	**GLP**	**Study Number / Location in eCTD**
28-day dose range-finding toxicity	Sprague Dawley rats	Subcutaneous injection	0 (mPD), 2, 10, 20, 30 (in mPD), 30 (in saline)	No	RM-493-TOX-026 / 4.2.3.2-1
28-day dose range-finding toxicity	Sprague Dawley rats	Subcutaneous injection	0 (mPD), 1, 3, 5 (in mPD), 2.5, 5, 7.5, 15, 30 (in saline)	No	RM-493-TOX-036 / 4.2.3.2-2
26-week toxicity with 4-week recovery	Sprague Dawley rats	Subcutaneous injection	0 (saline), 0 (mPD), 3, 15 (in saline), 0.5, 1.5, 3 (in mPD)	Yes	RM-493-TOX-023 / 4.2.3.2-3
13-week toxicity with 4-week recovery	Sprague Dawley rats	Continuous subcutaneous infusion	0 (saline), 10, 30, 60 (in saline)	Yes	RM-493-TOX-016 / 4.2.3.7.7-1
28-day dose range-finding toxicity	Cynomolgus monkeys	Subcutaneous injection	0 (saline), 0 (mPD), 0.2, 0.75, 2 (in mPD), 2 (in saline)	No	RM-493-TOX-027 / 4.2.3.2-4
39-week toxicity with 4-week recovery	Cynomolgus monkeys	Subcutaneous injection	0 (saline), 0 (mPD), 0.5/0.25, 1.5/0.5, 3/1 (in mPD), 3 (in saline)	Yes	RM-493-TOX-024 / 4.2.3.2-5
13-week toxicity with 10-day recovery	Cynomolgus monkeys	Continuous subcutaneous infusion	0 (saline), 1, 10, 25 (in saline)	Yes	RM-493-TOX-015 / 4.2.3.7.7-2
28-day dose range-finding (carcinogenicity)	rasH2 mice, wild-type	Subcutaneous injection	0 (saline), 0 (mPD), 10, 20 (in mPD)	Yes	RM-493-TOX-041 / 4.2.3.4.2-2
Female fertility/ EFD	Sprague Dawley rat	Subcutaneous injection	0 (saline), 0 (mPD), 0.5, 3, 5 (in mPD)	Yes	RM-493-TOX-029 / 4.2.3.5.1-1
Dose range-finding EFD	NZW rabbits	Subcutaneous injection	0 (mPD), 0.05, 0.5, 3, 10, 20 (in mPD)	No	RM-493-TOX-030 / 4.2.3.5.2-1
EFD	NZW rabbits	Subcutaneous injection	0 (mPD), 0.05, 0.1, 0.2 (in mPD)	Yes	RM-493-TOX-031 / 4.2.3.5.2-2
Pre/postnatal development	Sprague Dawley rat	Subcutaneous injection	0 (saline), 0 (mPD), 0.5, 3, 5 (in mPD)	Yes	RM-493-TOX-033 / 4.2.3.5.3-1
Juvenile dose range-finding	Sprague Dawley rats	Subcutaneous injection	0 (mPD), 1, 2.5, 5, (in mPD), 15 (in saline)	No	RM-493-TOX-034 / 4.2.3.5.4-1
Juvenile toxicity	Sprague Dawley rats	Subcutaneous injection	0 (saline), 0 (mPD), 0.5, 1.5, 3 (in mPD), 15 (in saline)	Yes	RM-493-TOX-035 / 4.2.3.5.4-2

EFD, embryo-fetal development; GLP, Good Laboratory Practice; mPD, mPEG-DSPE (N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine); NZW, New Zealand white.

2.6.7.3 トキシコキネティクス：トキシコキネティクス試験成績の一覧

Test Article: Setmelanotide (RM-493)

Daily Dose (mg/kg)	**Steady-State AUC (ng*hr/mL)[a]**									
	Mice		**Rats**		**Rabbits**		**Monkeys**		**Humans**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
0.025									268	
0.05					NA	199, 162				
					NA	214, 206[b]				
0.1					NA	524, 436[b]				
0.2					NA	941, 862[b]	1,420	1,360		
0.25							5,990[c]	3,730[c]		
0.5			1,480[d]	325[d]	NA	2,250, 1,380	3,820[c]	3,200[c]		
			NA	388[e]						
			NA	231, 247[f]						
			583[g]	295[g]						
0.75							5,220	5,420		
1			1,360				8,890[c]	21,600[c]		
			730[h]	753[h]			7,127*[i]	7,074*[i]		
							9,218*[ij]	9,354*[ij]		
1.5			2,930[d]	1,300[d]						
			2,020[g]	1,500[g]						
2			4,200	3,300			15,100	14,600		
							16,900*	16,500*		
2.5			2,950*				20,930*[i]	15,340*[i]		
			1,740[h]	2,100[h]						

									Test Article: Setmelanotide (RM-493)	
Daily Dose (mg/kg)	**Steady-State AUC (ng*hr/mL)[a]**									
	Mice		**Rats**		**Rabbits**		**Monkeys**		**Humans**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
3			4,550		NA	22,100, 12,400	21,400*[c]	22,800*[c]		
			5,730*[d]	2,440*[d]						
			6,160[d]	4,040[d]						
			1570[†]	NA						
			NA	3,150[e]						
			NA	2,050, 2,340[f]						
			3,870[g]	3,760[g]						
5			7,790							
			6,900*							
			NA	6,450[e]						
			NA	4,140, 4,340[f]						
			4,750[h]	5,940[h]						
7.5			11,800*							
10			19,600	16,200	NA	104,000, 61,600	54,390*[i]	82,980*[i]		
			29,990*[i]	24,850*[i]			51,430*[ij]	52,620*[ij]		
	11,800[k]	9,030[k]	33,520*[i]	41,610*[i]						
	10,400[k]	8,850[k]	80,660*[ij]	66,890*[ij]						
			NA	20,540*[i]						
12							73,400*[i]	82,200*[i]		
15			24,500*							
			28,300*[d]	28,700*[d]						
			12,400*[h]	10,000*[h]						
			6,660†	NA						
			19,500*[g]	18,100*[g]						
20	24,300[k]	15,600[k]	27,200	32,000	NA	131,000, 104,000				

Test Article: Setmelanotide (RM-493)										
Daily Dose (mg/kg)	**Steady-State AUC (ng*hr/mL)[a]**									
	Mice		**Rats**		**Rabbits**		**Monkeys**		**Humans**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
25					NA	158,200*[i]	120,400*[i]	157,200*[i]		
							183,800*[i]	150,700*[i]		
							154,100*[ij]	141,200*[ij]		
30			46,000*[i]	31,100*[i]						
			37,100	41,100						
			94,950*	96,680*						
			NA	73,670*[i]						
			139,800*[ij]	131,000*[ij]						
50			152,800*[i]	84,300*[i]						
60			182,500*[i]	179,200*[i]						
			277,100*[ij]	209,700*[ij]						
			NA	125,000*[i]						
100			217,700*[i]	227,200*[i]	NA	560,700*[i]				
			NA	166,600*[i]						
120			325,800*[i]	285,200*[i]						

AUC, area under the plasma concentration-time curve; NA, not applicable

a AUC over 24-hour dosing interval following once daily administration by subcutaneous injection or continuous subcutaneous infusion. Data shown as a single value are for males and females combined unless noted otherwise.

b Fertility and embryonic development study; AUC data from Gestation Day 13 and 17, respectively (Study RM-493-TOX-031 [4.2.3.5.2-2]).

c 39-week toxicity study. Doses for RM-493/mPEG-DSPE formulations were reduced from 0.5, 1.5, and 3 mg/kg/day to 0.25, 0.5, and 1 mg/kg/day, respectively, during Week 8 of treatment due to injection site reactions; AUC data from Week 39 (Study RM-493-TOX-024 [4.2.3.2-5]).

d 26-week toxicity study; AUC data from Day 180 (Study RM-493-TOX-023 [4.2.3.2-3]).

e Fertility and embryonic development study; AUC data from Gestation Day 17 (Study RM-493-TOX-029 [4.2.3.5.1-1]).

f Pre-/postnatal development study; AUC data from Gestation Day 20 and Lactation Day 10, respectively (Study RM-493-TOX-033 [4.2.3.5.3-1]).

g Juvenile rats treated from Postnatal Days 7 to 55; AUC data from Postnatal Day 55 (Study RM-493-TOX-035 [4.2.3.5.4-2]).

h Juvenile rats treated from Postnatal Days 7 to 34; AUC data from Postnatal Day 34 (Study RM-493-TOX-034 [4.2.3.5.4-1]).

i RM-493 in saline administered by continuous subcutaneous infusion 24 h/day. All other values are for RM-493 administered by once daily subcutaneous injection.

j 13-week toxicity study with administration by continuous subcutaneous infusion 24 h/day; AUC data from Day 91 (rats: Study RM-493-TOX-016 [4.2.3.7.7-1]; monkeys: Study RM-493-TOX-015 [4.2.3.7.7-2]).

k 28-day dose range-finding study in wild-type rasH2 mice; AUC data from Day 28 (Study RM-493-TOX-041 [4.2.3.4.2-2]).

Note: * RM-493 in saline; † RM-493 in Camurus FluidCrystal®; All other values are for RM-493 in mPEG-DSPE.

2.6.7.4 毒性試験：被験物質（バッチごと）一覧

Test Article: Setmelanotide (RM-493)													
Batch No.[a]	**Purity (%)[b]**	**Specified Impurities (Area %)**										**Study Number / Location in eCTD**	**Type of Study**
		0.88[c]	**0.91**	**0.96**	**0.98**	**0.99**	**1.05**	**1.13**	**1.17**	**1.28**	**1.51**		
PROPOSED SPECIFICATION:	**≥75 (net peptide)**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**		
[redacted]	79	0.24	0.35	0.16	0.29	0.20	0.24	0.16	0.16	0.11	0.24	RM-493-TOX-035 / 4.2.3.5.4-2	7-week SC injection toxicity study in juvenile rats
												RM-493-TOX-023 / 4.2.3.2-3	26-week SC injection toxicity in rats
												RM-493-TOX-016 / 4.2.3.7.7-1	13-week continuous SC infusion toxicity in rats
												RM-493-TOX-015 / 4.2.3.7.7-2	13-week continuous SC infusion toxicity in monkeys
[redacted] (used to formulate DP Lot [redacted])[d]	78.6	0.13	0.06	0.08	0.23	ND	ND	0.18	0.17	0.30	0.15	RM-493-TOX-035 / 4.2.3.5.4-2	7-week SC injection toxicity study in juvenile rats
												RM-493-TOX-023 / 4.2.3.2-3	26-week SC injection toxicity in rats
												RM-493-TOX-029 / 4.2.3.5.1-1	SC injection fertility and embryo-fetal development in female rats
[redacted] (used to formulate DP Lot [redacted])[d]	78.6	0.13	ND	0.08	0.23	ND	ND	0.18	0.17	0.30	0.15	RM-493-TOX-026 / 4.2.3.2-1	28-day SC injection dose range-finding study in rats
												RM-493-TOX-036 / 4.2.3.2-2	28-day SC injection dose range-finding study in rats

Test Article: Setmelanotide (RM-493)													
Batch No.[a]	**Purity (%)[b]**	**Specified Impurities (Area %)**										**Study Number / Location in eCTD**	**Type of Study**
		0.88[c]	**0.91**	**0.96**	**0.98**	**0.99**	**1.05**	**1.13**	**1.17**	**1.28**	**1.51**		
PROPOSED SPECIFICATION:	**≥75 (net peptide)**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**		
												RM-493-TOX-034 / 4.2.3.5.4-1	28-day SC injection dose range-finding study in juvenile rats
												RM-493-TOX-027 / 4.2.3.2-4	28-day SC injection dose range-finding study in monkeys
												RM-493-TOX-024 / 4.2.3.2-5	39-week SC injection toxicity in monkeys
												RM-493-TOX-030 / 4.2.3.5.2-1	SC injection dose range-finding embryo-fetal development in rabbits
												RM-493-TOX-031 / 4.2.3.5.2-2	SC injection embryo-fetal development in rabbits
												RM-493-TOX-033 / 4.2.3.5.3-1	SC injection pre- and postnatal development in rats
[redacted]	77	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	RM-493-TOX-008 / 4.2.3.3.1-1	Bacterial reverse mutation
												RM-493-TOX-009 / 4.2.3.3.1-2	Chromosome aberration
[redacted]	80	0.29	0.19	0.17	0.12	ND	0.13	0.15	0.20	0.14	0.23	RM-493-TOX-020 / 4.2.3.3.2-1	Bone marrow micronucleus in rats

Test Article: Setmelanotide (RM-493)													
Batch No.[a]	**Purity (%)[b]**	**Specified Impurities (Area %)**										**Study Number / Location in eCTD**	**Type of Study**
		0.88[c]	**0.91**	**0.96**	**0.98**	**0.99**	**1.05**	**1.13**	**1.17**	**1.28**	**1.51**		
PROPOSED SPECIFICATION:	**≥75 (net peptide)**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**		
[redacted] (used to formulate DP Lot [redacted]) [d]	79	0.09	ND	0.09	0.15	0.12	0.06	0.27	0.05	ND	0.08	RM-493-TOX-040 / 4.2.3.4.2-1	7-day SC injection dose range-finding study in wild type rasH2 mice
												RM-493-TOX-041 / 4.2.3.4.2-2	28-day SC injection dose range-finding study in wild type rasH2 mice
												RM-493-TOX-042 / 4.2.3.4.2-3	26-week SC injection carcinogenicity study in Tg.rasH2 mice
[redacted] (used to formulate DP Lots [redacted])	83	0.1	<0.05	0.1	0.1	0.1	0.1	0.20	0.1	<0.05	0.1	RM-493-TOX-042 / 4.2.3.4.2-3 RM-493-TOX-044 / 4.2.3.4.2-4 RM-493-TOX-045 / 4.2.3.4.2-5	26-week SC injection carcinogenicity studies in Tg.rasH2 mice
[redacted] (used to formulate DP Lots [redacted])	84	0.1	<0.05	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	RM-493-TOX-044 / 4.2.3.4.2-4 RM-493-TOX-045 / 4.2.3.4.2-5	26-week SC injection carcinogenicity studies in Tg.rasH2 mice
[redacted] (used to formulate DP Lot [redacted])	83	0.1	<0.05	0.1	0.1	0.1	0.1	0.20	0.1	<0.05	0.1	RM-493-TOX-045 / 4.2.3.4.2-5	26-week SC injection carcinogenicity study in Tg.rasH2 mice
[redacted] [d]	100	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	RM-493-TOX-022 / 4.2.3.6-1	7-day local SC irritation study in rats

DP, drug product; NA, not applicable due to use of different analytical method.; ND, not detected; SC, subcutaneous
a Batch numbers are for drug substance unless footnoted as drug product.
b % of nominal concentration or mg/mL for drug product batches if indicated.
c Relative retention time.
d RM-493 in mPEG-DSPE; drug product certificates of analysis are included in study reports.

2.6.7.5 単回投与毒性試験

Setmelanotide の単回投与毒性試験は実施していない。

2.6.7.6 反復投与毒性試験：重要な試験以外の試験

Test Article: Setmelanotide (RM-493)							
Species/ Strain	**Method of Administration (Vehicle)**	**Duration of Dosing**	**Doses (mg/kg/day)[a]**	**Gender and No. Per Group**	**NOAEL (mg/kg/day)**	**Noteworthy Findings**	**Study Number / Location in eCTD**
Sprague Dawley rat	SC injection (mPD[b], saline)	28 days	0 (mPD), 2, 10, 20, 30 (10 mg/mL in mPD); 30 (10 mg/mL in saline)	Main:5/sex TK: 12/sex	NA	All groups including vehicle control: Red/black/scabbed skin and lesions, eschar, eschar exfoliation, blanching, and/or necrosis at injection sites; increased white blood cell counts and fibrinogen; early termination from Days 16 to 23.	RM-493-TOX-026 / 4.2.3.2-1
Sprague Dawley rat	SC injection (mPD[c], saline)	28 days	0 (mPD), 1, 3, 5 (1 mg/mL in mPD); 2.5 (2.5 mg/mL in saline); 5 (5 mg/mL in saline); 7.5, 15, 30 (7.5 mg/mL in saline)	Main:5/sex TK: 12/sex	NA	All groups including vehicle control: Injection site changes including clinical observations of erythema, edema, eschar, eschar exfoliation and/or blanching, macroscopic observations of swelling, thick and/or firm, and microscopic changes of degeneration/ necrosis of the subcutis and myofiber, subcutaneous edema, mixed inflammatory cell infiltration, fibrosis, vascular inflammation and/or thrombosis.	RM-493-TOX-036 / 4.2.3.2-2
Cynomolgus monkey	SC injection (mPD[b], saline)	28 days	0 (mPD); 0.2, 0.75, 2 (10 mg/mL in mPD); 2 (10 mg/mL in saline)	3/sex	2 (in mPD and saline)	All mPD groups including control: subcutaneous inflammation and infiltration of vacuolated histiocytes and multinucleated giant cells at injection sites. ≥0.2: Discoloration of the skin (black skin on muzzle and cranium) correlating microscopically with increased pigmentation of the skin and injection site, with no evidence of melanocyte proliferation.	RM-493-TOX-027 / 4.2.3.2-4

mPD, mPEG-DSPE; NA, not applicable; SC, subcutaneous; TK, toxicokinetic

a Setmelanotide concentration in dose formulations are shown in parenthesis.

b Undiluted clinical formulation of mPEG-DSPE vehicle.

c Clinical formulation of mPEG-DSPE diluted 10-fold with 5% mannitol in sterile water for injection, USP.

2.6.7.7 反復投与毒性試験：重要な試験

2.6.7.7.1 ラットを用いた 26 週間皮下投与毒性試験及び 4 週間回復性試験

Report Title: A 26-Week Subcutaneous Injection Toxicity Study of RM-493 in the Sprague Dawley Rat with a 4-Week Recovery Period		**Test Article:** Setmelanotide (RM-493)
Species/Strain: Rat/Sprague Dawley	**Duration of Dosing**: 26 Weeks	**Study No.** RM-493-TOX-023 **Location in eCTD:** 4.2.3.2-3
Initial Age: 10 weeks	**Duration of Postdose**: 4 Weeks	
Date of First Dose: 08 Mar 2016	**Method of Administration**: Subcutaneous Injection	**GLP Compliance**: Yes
	Vehicle/Formulation: Reference Item (RI) 1: Saline Reference Item (RI) 2: mPEG-DSPE vehicle (clinical formulation diluted 10-fold with 5% mannitol in sterile water for injection, USP).	
Special Features: Clinical setmelanotide/mPEG-DSPE diluted 10-fold in 5% mannitol in sterile water for injection, USP; male reproductive evaluations, local irritation, anti-drug antibody, aldosterone, and KIM-1 evaluations		
No Observed Adverse Effect Level: 15 mg/kg/day RM-493 in saline; 3 mg/kg/day RM-493 in mPEG-DSPE		

Dose (mg/kg)	**0 (Saline)**		**0 (mPEG-DSPE)**		**3 (Saline)**		**15 (Saline)**		**0.5 (mPEG-DSPE)**		**1.5 (mPEG-DSPE)**		**3 (mPEG-DSPE)**	
Number of Animals:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main:	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**
Recovery:	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Toxicokinetic:	**3**	**3**	**3**	**3**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**
Toxicokinetics														
Day 1														
$AUC_{0-\tau}$ (ng*hr/mL)	BLQ	BLQ	BLQ	BLQ	1,370	877	5,000	4,420	384	295	1,330	858	2,730	1,520
C_{max} (ng/mL)	BLQ	BLQ	BLQ	BLQ	636	460	1,830	1,110	158	108	379	247	599	376
Day 90														
$AUC_{0-\tau}$ (ng*hr/mL)	BLQ	BLQ	BLQ	BLQ	5,600	2,310	29,200	23,900	571	524	3,090	2,210	5,830	5,820
C_{max} (ng/mL)	BLQ	BLQ	BLQ	BLQ	907	1,040	4,910	6,230	145	157	532	591	912	1,110
Day 180														
$AUC_{0-\tau}$ (ng*hr/mL)	BLQ	BLQ	BLQ	BLQ	5,730	2,440	28,300	28,700	1,480	325	2,930	1,300	6,160	4,040
C_{max} (ng/mL)	BLQ	BLQ	BLQ	BLQ	998	1,070	3,820	4,940	156	121	417	314	644	901

Report Title: A 26-Week Subcutaneous Injection Toxicity Study of RM-493 in the Sprague Dawley Rat with a 4-Week Recovery Period											**Test Article:** Setmelanotide (RM-493)			
Dose (mg/kg)	**0 (Saline)**		**0 (mPEG-DSPE)**		**3 (Saline)**		**15 (Saline)**		**0.5 (mPEG-DSPE)**		**1.5 (mPEG-DSPE)**		**3 (mPEG-DSPE)**	
Number of Animals:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main:	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**
Recovery:	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Noteworthy Findings														
Died or Euthanized Moribund	-	-	-	1	4	-	2	-	1	1	1	1	-	-
Body Weight[a]	750 g	375 g	-12C	-6	-8A	-11C	-18C	-15C	-10B	-14CD	-14C	-10C	-16C	-14CD
Food Consumption	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ophthalmology	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clinical Observations														
Injection site														
Skin, Scab	7	11	19	19	5	5	19	11	8	9	7	5	13	8
Fur, Thin Cover	11	19	20	20	16	19	20	20	14	19	17	20	16	19
Skin, Lesions	0	0	11	3	0	1	7	0	0	1	0	2	0	1
Skin, Lesions w/discharge	0	0	1	0	0	0	2	0	0	0	0	0	0	1
Skin, Thickening	0	0	0	0	20	19	20	20	20	20	20	17	20	20
Hematology														
Absolute neutrophil count (10^3/uL)	1.934	1.001	2.780A	1.673E	2.065	1.204	2.690	1.301D	2.313	1.023G	2.848A	1.058G	3.002B	1.344A
Coagulation														
Fibrinogen (mg/dL)														
Week 13	320.0	210.3	377.1B	258.6B	474.4C	230.3	514.7C	256.3B	489.1CL	217.4A	489.3CL	231.8	492.1CL	231.4
Week 26	354.7	234.1	428.9B	260.5	488.8C	209.3	570.8C	251.6	485.2CJ	208.9C	473.4C	209.9C	480.6CJ	226.9A

Report Title: A 26-Week Subcutaneous Injection Toxicity Study of RM-493 in the Sprague Dawley Rat with a 4-Week Recovery Period											**Test Article:** Setmelanotide (RM-493)			
Dose (mg/kg)	**0 (Saline)**		**0 (mPEG-DSPE)**		**3 (Saline)**		**15 (Saline)**		**0.5 (mPEG-DSPE)**		**1.5 (mPEG-DSPE)**		**3 (mPEG-DSPE)**	
Number of Animals:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main:	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**
Recovery:	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Serum Chemistry														
Albumine (g/dL)														
Week 13	4.06	5.40	3.83B	5.23	3.75C	5.67	3.63C	5.45	3.76C	5.58	3.55CL	5.42	3.47CL	5.39
Day 183 to 186	4.07	5.95	3.93	5.61	3.91	5.89	3.71C	5.63	3.85A	5.87	3.82A	5.86	3.78B	5.95
A/G ratio														
Week 13	1.46	2.33	1.45	2.29	1.30E	2.41	1.25F	2.26	1.27FI	2.34	1.31DG	2.32	1.30EG	2.26
Day 183 to 186	1.43	2.45	1.36	2.26	1.35	2.44	1.23C	2.31	1.32	2.49	1.36	2.46	1.36	2.35
Glucose (mg/dL)														
Week 13	110.0	97.2	96.6B	101.6	97.5B	100.8	90.1C	99.2	104.5	100.4	103.0	101.5	95.5C	102.6
Day 183 to 186	109.8	104.0	96.6A	100.1	107.4	93.3	96.0A	96.9	103.2	98.7	102.1	96.6	94.8A	98.4
Cholesterol (mg/dL)														
Week 13	91.7	109.9	96.7	104.7	77.9A	101.4	69.9C	99.3	88.6	94.5	83.6	99.7	97.0	108.2
Day 183 to 186	106.7	131.2	112.2	136.6	94.1	121.3	82.9A	120.2	111.0	113.5D	104.5	129.4	119.1	141.9
Triglycerides (mg/dL)														
Week 13	95.1	54.7	82.5	43.6	58.5E	41.6	56.2E	44.1	80.4	42.5	48.7FI	37.2	57.8EH	39.4
Day 183 to 186	127.9	81.1	69.8D	69.0	73.1D	49.9	51.1F	45.0	89.6	58.7	63.6E	56.1	70.7D	50.3
Aldosterone (pg/mL)														
Day 183	215.69	480.23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	74.71 D	458.33
Anti-Drug Antibody	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urinalysis														
Specific Gravity														
Week 13	1.0326	1.0364	1.0241	1.0336	1.0221	1.0221E	1.0286	1.0205E	1.0253	1.0214E	1.0247	1.0218D	1.0228	1.0191FG
Day 183 to 186	1.0347	1.0344	1.0311	1.0365	1.0261	1.0245	1.0326	1.0262	1.0305	1.0232D	1.0323	1.0249	1.0251	1.0219E
KIM-1 (ng/mL)														
Week 27	0.061	0.105	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.059	0.106

Report Title: A 26-Week Subcutaneous Injection Toxicity Study of RM-493 in the Sprague Dawley Rat with a 4-Week Recovery Period											**Test Article:** Setmelanotide (RM-493)			
Dose (mg/kg)	**0 (Saline)**		**0 (mPEG-DSPE)**		**3 (Saline)**		**15 (Saline)**		**0.5 (mPEG-DSPE)**		**1.5 (mPEG-DSPE)**		**3 (mPEG-DSPE)**	
Number of Animals:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main:	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**
Recovery:	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Male Reproductive Assessments														
Sperm Motility, Concentration, Morphology	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA
Stage-Dependent Spermatogenesis (testicular histopathology)	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA
Parental Performance	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA
Ovarian/Uterine Evaluation	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-
Organ Weight[b]														
Gland Prostate	1.538g	NA	-5	NA	30	NA	16	NA	21BL	NA	19BL	NA	17AK	NA
Gross Pathology[c]														
Number of animals examined	15	15	15	14	14	15	14	15	15	15	14	14	15	15
Injection Site														
Focus; dark	7	3	14	11	10	11	13	14	7	2	12	3	13	3
Focus; pale	0	0	0	11	0	0	0	9	0	0	0	2	0	11
Thin hair coat	0	0	5	3	0	0	10	3	0	0	0	0	1	0
Scab; dark	0	0	7	0	0	0	10	1	0	0	1	0	0	1
Thick	0	0	15	9	10	1	12	4	13	0	12	3	15	10
Skin														
Scab; dark	0	NA	4	NA	0	NA	4	NA	0	NA	0	NA	0	NA
Scab; pale/dark	0	0	0	4	0	0	0	0	0	0	0	0	0	0

Report Title: A 26-Week Subcutaneous Injection Toxicity Study of RM-493 in the Sprague Dawley Rat with a 4-Week Recovery Period											**Test Article:** Setmelanotide (RM-493)			
Dose (mg/kg)	**0 (Saline)**		**0 (mPEG-DSPE)**		**3 (Saline)**		**15 (Saline)**		**0.5 (mPEG-DSPE)**		**1.5 (mPEG-DSPE)**		**3 (mPEG-DSPE)**	
Number of Animals:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main:	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**
Recovery:	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Histopathology[c]														
Number of animals examined	15	15	15	14	14	15	14	15	15	15	14	14	15	15
Injection Sites														
Infiltration mixed cell														
Minimal	5	0	0	0	11	14	0	8	7	15	1	14	0	4
Mild	0	0	9	14	0	0	14	7	8	0	11	0	12	10
Moderate	0	0	6	0	0	0	0	0	0	0	2	0	3	1
Hemorrhage														
Minimal	2	2	10	7	5	8	8	8	7	1	9	3	10	3
Mild	0	0	4	4	0	0	5	5	0	0	3	0	2	1
Moderate	0	0	1	0	0	1	0	0	0	0	0	0	1	0
Fibrosis														
Minimal	2	1	0	1	12	7	0	7	9	1	1	13	0	4
Mild	0	0	1	3	0	0	2	3	4	0	3	0	0	3
Moderate	0	0	11	10	0	0	12	1	0	0	10	0	15	7
Marked	0	0	3	0	0	0	0	0	0	0	0	0	0	0
Infiltration, vacuolated histiocytes														
Minimal	0	0	1	0	0	0	0	0	6	15	2	7	1	5
Mild	0	0	14	14	0	0	0	0	8	0	12	6	14	10
Degeneration/ regeneration, myofiber														
Minimal	0	0	6	8	1	2	1	9	1	0	5	2	2	6
Mild	0	0	5	1	0	0	3	3	0	0	0	1	1	1
Moderate	0	0	1	0	0	0	10	3	0	0	0	0	0	0

Report Title: A 26-Week Subcutaneous Injection Toxicity Study of RM-493 in the Sprague Dawley Rat with a 4-Week Recovery Period											**Test Article:** Setmelanotide (RM-493)			
Dose (mg/kg)	**0 (Saline)**		**0 (mPEG-DSPE)**		**3 (Saline)**		**15 (Saline)**		**0.5 (mPEG-DSPE)**		**1.5 (mPEG-DSPE)**		**3 (mPEG-DSPE)**	
Number of Animals:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main:	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**
Recovery:	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Atrophy														
Minimal	0	0	2	7	0	0	9	3	0	0	0	0	0	0
Mild	0	0	0	0	0	0	4	0	0	0	0	0	0	0
Inflammation, skin														
Minimal	0	1	3	5	0	0	2	2	0	0	0	1	2	3
Mild	0	0	2	0	0	0	7	0	0	0	1	0	0	0
Moderate	0	0	0	0	0	0	3	0	0	0	1	0	0	0
Erosion/ulcer, skin														
Minimal	0	0	0	0	0	0	0	1	0	1	0	0	1	2
Mild	0	0	2	1	0	0	6	1	0	0	0	0	1	0
Moderate	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Marked	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Necrosis, skin														
Mild	0	NA	1	NA	0	NA	0	NA	0	NA	0	NA	0	NA
Moderate	0	NA	1	NA	0	NA	1	NA	0	NA	0	NA	0	NA
Inflammation, vascular														
Minimal	0	0	0	1	0	0	1	2	0	0	0	0	0	0
Mild	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Muscle, Skeletal														
Degeneration /regeneration														
Minimal	0	0	0	0	0	0	0	4	0	1	1	1	0	0
Mild	0	0	0	0	0	0	3	3	0	0	0	0	0	0

Report Title: A 26-Week Subcutaneous Injection Toxicity Study of RM-493 in the Sprague Dawley Rat with a 4-Week Recovery Period											**Test Article:** Setmelanotide (RM-493)			
Dose (mg/kg)	**0 (Saline)**		**0 (mPEG-DSPE)**		**3 (Saline)**		**15 (Saline)**		**0.5 (mPEG-DSPE)**		**1.5 (mPEG-DSPE)**		**3 (mPEG-DSPE)**	
Number of Animals:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main:	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**
Recovery:	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Brain														
Vacuolation, choroid plexus														
Minimal	0	0	0	2	0	0	0	0	1	1	3	0	0	2
Post-dose Evaluation (Recovery)														
Hematology														
Absolute neutrophil count (10^3/uL)	2.143	1.030	2.774	1.488	3.077	1.438	4.045	1.053	2.235	1.260	1.755	1.136	1.354	1.038
Coagulation														
Fibrinogen (mg/dL)	355.5	238.8	378.6	206.4	336.7	243.0	452.6	218.0	360.5	239.3	340.7	222.0	311.0	225.0
Serum Chemistry														
Albumine (g/dL)	4.24	5.98	4.34	5.96	4.40	5.88	4.10	5.80	4.38	5.63	4.20	5.78	4.13	5.86
A/G ratio	1.46	2.40	1.40	2.40	1.63	2.48	1.40	2.48	1.55	2.13	1.48	2.36	1.67A	2.46
Glucose (mg/dL)	113.4	97.8	95.6	102.8	104.0	103.0	110.0	111.0	111.8	93.5	109.6	107.6	106.0	105.2
Cholesterol (mg/dL)	119.0	140.0	111.2	134.2	91.7	142.0	87.6A	124.6	84.5A	141.5	109.2	118.2	77.4BJ	118.6
Triglycerides (mg/dL)	167.4	85.4	107.4	58.2	188.7	84.2	165.6	103.4	153.5	75.8	122.8	135.6	118.7	81.4
Urinalysis														
Specific Gravity	1.0294	1.0374	1.0296	1.0460	1.0393	1.0292	1.0412	1.0302	1.0460	1.0350	1.0484	1.0304	1.0324	1.0258
Organ Weight[b]														
Gland Prostate	1.548g	NA	1	NA	-1	NA	19	NA	1	NA	1	NA	6	NA

Report Title: A 26-Week Subcutaneous Injection Toxicity Study of RM-493 in the Sprague Dawley Rat with a 4-Week Recovery Period											**Test Article:** Setmelanotide (RM-493)			
Dose (mg/kg)	**0 (Saline)**		**0 (mPEG-DSPE)**		**3 (Saline)**		**15 (Saline)**		**0.5 (mPEG-DSPE)**		**1.5 (mPEG-DSPE)**		**3 (mPEG-DSPE)**	
Number of Animals:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main:	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**
Recovery:	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Gross Pathology[c]														
Number of animals examined	5	5	5	5	3	5	5	5	5	4	5	5	5	5
Injection Site														
Abrasion/ scab; pale/dark	0	NA	0	NA	0	NA	3	NA	0	NA	0	NA	0	NA
Thin hair coat	NA	0	NA	4	NA	0	NA	1	NA	1	NA	1	NA	1
Scab; dark	NA	0	NA	1	NA	0	NA	0	NA	0	NA	0	NA	0
Thick	1	0	5	4	1	0	4	0	3	0	5	3	5	5
Histopathology[c]														
Number of animals examined	5	5	5	5	3	5	5	5	5	4	5	5	5	5
Injection Site														
Infiltration mixed cell														
Minimal	1	0	2	1	2	0	1	0	0	0	2	1	0	2
Mild	0	NA	1	NA	0	NA	0	NA	0	NA	0	NA	0	NA
Hemorrhage														
Minimal	0	0	1	1	1	0	1	0	0	0	2	1	0	1
Mild	NA	0	NA	1	NA	0	NA	0	NA	0	NA	0	NA	0
Fibrosis														
Minimal	0	0	0	2	0	0	0	4	5	0	2	4	0	2
Mild	0	0	2	3	0	0	5	0	0	0	3	0	3	3
Moderate	0	NA	3	NA	0	NA	0	NA	0	NA	0	NA	2	NA

Report Title: A 26-Week Subcutaneous Injection Toxicity Study of RM-493 in the Sprague Dawley Rat with a 4-Week Recovery Period											**Test Article:** Setmelanotide (RM-493)			
Dose (mg/kg)	**0 (Saline)**		**0 (mPEG-DSPE)**		**3 (Saline)**		**15 (Saline)**		**0.5 (mPEG-DSPE)**		**1.5 (mPEG-DSPE)**		**3 (mPEG-DSPE)**	
Number of Animals:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main:	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**
Recovery:	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Infiltration, vacuolated histiocytes														
Minimal	0	0	3	5	0	0	0	0	0	2	1	5	5	5
Mild	0	NA	2	NA	0	NA	0	NA	0	NA	0	NA	0	NA
Atrophy; hair follicle														
Minimal	0	0	1	4	0	0	2	2	0	0	0	0	0	1
Mild	0	0	0	1	0	0	1	0	0	0	0	0	0	0
Erosion/ulcer; skin														
Minimal	0	2	0	2	0	0	0	1	1	0	0	0	0	0
Moderate	0	NA	0	NA	0	NA	3	NA	0	NA	0	NA	0	NA
Brain														
Vacuolation, choroid plexus														
Minimal	0	0	0	0	0	0	0	0	0	2	0	2	0	0

-, no noteworthy findings; $AUC_{0-\tau}$, area under the plasma concentration-time curve during a time interval; BLQ, below the limit of quantitation; C_{max}, maximum plasma concentration; F, female; GLP, Good Laboratory Practice; KIM-1, kidney injury molecule-1; M, male; NA, not applicable; RI, reference item

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

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

c Number of animals with finding.

Significantly different from control group (Group 1) value: A, $p \leq 0.05$; B, $p \leq 0.01$; C, $p \leq 0.001$ (T-test); D, $p \leq 0.05$; E, $p \leq 0.01$; F, $p \leq 0.001$ (Wilcoxon).

Significantly different from control (Group 2) value: G, $p \leq 0.05$; H, $p \leq 0.01$; I, $p \leq 0.001$ (Wilcoxon); J, $p \leq 0.05$; K, $p \leq 0.01$; L, $p \leq 0.001$ (T-test).

2.6.7.7.2 サルを用いた 39 週間皮下投与毒性試験及び 4 週間回復性試験

Report Title: A 39-Week Subcutaneous Injection Toxicity Study of RM 493 in Cynomolgus Monkey with a 4 Week Recovery period.		**Test Article:** Setmelanotide (RM-493)
Species/Strain: Monkey/Cynomolgus	**Duration of Dosing**: 39 Week	**Study No.** RM-493-TOX-024 **Location in eCTD:** 4.2.3.2-5
Initial Age: 1.5 to 5 years	**Duration of Postdose**: 4 Week	
Date of First Dose: 19 Jan 2016	**Method of Administration**: Subcutaneous Injection	**GLP Compliance**: Yes
	Vehicle/Formulation: mPEG-DSPE; Saline	
Special Features: Local irritation, anti-drug antibody, aldosterone, and blood pressure evaluations. Doses shown below for RM-493 in mPEG-DSPE are before/after dose reduction at Week 8 due to adverse injection site reactions (clinical setmelanotide/mPEG-DSPE and mPEG-DSPE vehicle formulations diluted 10-fold with 5% mannitol in sterile water for injection, USP at Week 8).		
No Observed Adverse Effect Level: 3 mg/kg/day		

Dose (mg/kg)	**0 (Saline)**		**0 (mPEG-DSPE)**		**3 (Saline)**		**0.5/0.25 (mPEG-DSPE)**		**1.5/0.5 (mPEG-DSPE)**		**3/1 (mPEG-DSPE)**	
Number of Animals:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main:	4	4	4	4	4	4	4	4	4	4	4	4
Recovery:	2	2	2	2	2	2	NA	NA	NA	NA	2	2
Toxicokinetics												
Day 1												
$AUC_{0-\tau}$ (ng*hr/mL)	BLQ	BLQ	BLQ	BLQ	19,600	25,100	6,540	3,070	9,450	8,730	20,000	20,300
C_{max} (ng/mL)	BLQ	BLQ	BLQ	BLQ	5,210	5,840	329	307	706	575	1,390	1,670
Week 13												
$AUC_{0-\tau}$ (ng*hr/mL)	BLQ	BLQ	BLQ	BLQ	16,700	20,900	3,280	1,790	3,960	2,410	6,920	8,250
C_{max} (ng/mL)	BLQ	BLQ	BLQ	BLQ	4,250	5,260	294	190	381	280	791	792
Week 26												
$AUC_{0-\tau}$ (ng*hr/mL)	BLQ	BLQ	BLQ	BLQ	16,400	21,400	5,620	4,150	3,570	2,760	7,940	21,100
C_{max} (ng/mL)	BLQ	BLQ	BLQ	BLQ	4,170	5,330	417	319	430	314	792	1,410
Week 39												
$AUC_{0-\tau}$ (ng*hr/mL)	BLQ	BLQ	BLQ	BLQ	21,400	22,800	5,990	3,730	3,820	3,200	8,890	21,600
C_{max} (ng/mL)	BLQ	BLQ	BLQ	BLQ	5,020	6,240	439	346	399	468	766	1,640

Report Title: A 39-Week Subcutaneous Injection Toxicity Study of RM 493 in Cynomolgus Monkey with a 4 Week Recovery period.											**Test Article:** Setmelanotide (RM-493)	
Dose (mg/kg)	**0 (Saline)**		**0 (mPEG-DSPE)**		**3 (Saline)**		**0.5/0.25 (mPEG-DSPE)**		**1.5/0.5 (mPEG-DSPE)**		**3/1 (mPEG-DSPE)**	
Number of Animals	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main:	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Recovery:	**2**	**2**	**2**	**2**	**2**	**2**	**NA**	**NA**	**NA**	**NA**	**2**	**2**
Noteworthy Findings												
Died or Euthanized Moribund	0	0	0	0	0	0	0	0	0	0	0	0
Body Weight[a]	3.97 kg	2.92 kg	-11	4	-24B	-9	-12	9	-13	4	-25B	-2
Food Consumption	-	-	-	-	-	-	-	-	-	-	-	-
Ophthalmology	-	-	-	-	-	-	-	-	-	-	-	-
Electrocardiography	-	-	-	-	-	-	-	-	-	-	-	-
Blood Pressure	-	-	-	-	-	-	-	-	-	-	-	-
Clinical Observations[b]												
Skin Black	0	0	0	0	337(6)	266(6)	159(4)	214(4)	227(4)	209(4)	313(6)	261(6)
Skin Thickening	0	0	8(4)	13(6)	0	0	3(1)	4(2)	12(3)	8(2)	30(6)	34(6)
Hematology	-	-	-	-	-	-	-	-	-	-	-	-
Coagulation												
Fibrinogen (mg/dL)	257.6	269.0	289.2	217.3	249.0	227.8	252.0	236.8	249.0	282.0	245.2	327.3B
Serum Chemistry	-	-	-	-	-	-	-	-	-	-	-	-
Anti – Drug Antibody	-	-	-	-	-	-	-	-	-	-	-	-
Urinalysis												
Specific Gravity												
Week 13	1.0265	1.0215	1.0230	1.0170	1.0205	1.0208	1.0243	1.0235	1.0193	1.0313G	1.0218	1.0170
Week 39	1.0273	1.0228	1.0230	1.0223	1.0223	1.0258	1.0263	1.0223	1.0263	1.0223	1.0238	1.0193
NAG(U/L)												
Week 13	1.97	4.72	2.70	3.67	3.42	3.47	3.70	4.33	2.73	3.65	2.67	4.35
Week 39	0.48	1.70	1.43	2.55	2.42D	5.98	1.83	3.00	3.40	1.95	0.88	5.05
NAG/CREAT(U/g)												
Week 39	0.82	2.30	3.02	3.45	4.40D	6.48	2.60	4.03	3.98D	3.28	1.82	6.73

Report Title: A 39-Week Subcutaneous Injection Toxicity Study of RM 493 in Cynomolgus Monkey with a 4 Week Recovery period.									**Test Article:** Setmelanotide (RM-493)			
Dose (mg/kg)	**0 (Saline)**		**0 (mPEG-DSPE)**		**3 (Saline)**		**0.5/0.25 (mPEG-DSPE)**		**1.5/0.5 (mPEG-DSPE)**		**3/1 (mPEG-DSPE)**	
Number of Animals	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main:	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Recovery:	**2**	**2**	**2**	**2**	**2**	**2**	**NA**	**NA**	**NA**	**NA**	**2**	**2**
Organ Weight	-	-	-	-	-	-	-	-	-	-	-	-
Gross Pathology[c]												
Number of animals examined	4	4	4	4	4	4	4	4	4	4	4	4
Skin												
Discoloration dark	-	-	-	-	4	4	2	4	4	4	3	4
Histopathology[c]												
Skin												
Pigment increased												
Minimal	-	-	-	-	1	2	1	2	1	3	1	1
Mild	-	-	-	-	3	1	2	2	3	0	1	3
Injection site												
Infiltration mixed cell												
Minimal	-	-	-	1	-	-	-	-	-	1	1	-
Mild	-	-	-	-	-	-	-	-	1	1	-	-
Infiltration mononuclear cell												
Minimal	-	-	1	1	1	-	-	-	-	-	-	-
Mild	-	-	1	-	-	-	-	-	-	-	-	2
Inflammation, chronic-active												
Minimal	-	-	-	-	-	1	-	-	-	1	-	-
Mild	-	-	-	1	-	-	-	-	-	-	-	3
Moderate	-	-	2	-	-	-	-	-	-	-	-	1
Brain												
Macrophage aggregation, choroid plexus												
Minimal	-	-	4	4	-	-	-	2	4	3	4	4

Report Title: A 39-Week Subcutaneous Injection Toxicity Study of RM 493 in Cynomolgus Monkey with a 4 Week Recovery period.											**Test Article:** Setmelanotide (RM-493)	
Dose (mg/kg)	**0 (Saline)**		**0 (mPEG-DSPE)**		**3 (Saline)**		**0.5/0.25 (mPEG-DSPE)**		**1.5/0.5 (mPEG-DSPE)**		**3/1 (mPEG-DSPE)**	
Number of Animals	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main:	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Recovery:	**2**	**2**	**2**	**2**	**2**	**2**	**NA**	**NA**	**NA**	**NA**	**2**	**2**
Post-Dose Evaluation (recovery)												
Body Weight (kg)[a]	3.95Z	3.20Z	-13Z	5Z	6Z	0Z	NA	NA	NA	NA	-10Z	-8Z
Coagulation												
Fibrinogen (mg/dL)	NA	210.0Z	238.0Z	200.5Z	183.0Z	195.0Z	NA	NA	NA	NA	197.5Z	282.5Z
Urinalysis												
Specific Gravity												
Week 43	1.0275Z	1.0190Z	1.0180Z	1.0250Z	1.0310Z	1.0260Z	NA	NA	NA	NA	1.0220Z	1.0150Z
NAG(U/L)												
Week 43	2.35Z	2.10Z	0.55Z	5.05Z	4.40Z	4.85Z	NA	NA	NA	NA	1.20Z	2.35Z
NAG/CREAT(U/g)												
Week 43	2.20Z	4.35Z	1.40Z	7.90Z	4.95Z	5.65Z	NA	NA	NA	NA	2.25Z	5.55Z
Gross Pathology[c]												
Number of animals examined	2	2	2	2	2	2	NA	NA	NA	NA	2	2
Skin												
Discoloration dark	0	0	0	0	2	1	NA	NA	NA	NA	1	1
Histopathology[c]												
Number of animals examined	2	2	2	2	2	2	NA	NA	NA	NA	2	2
Injection site												
Infiltration mixed cell												
Minimal	0	0	0	0	0	0	NA	NA	NA	NA	1	0
Infiltration mononuclear cell												
Minimal	0	0	0	2	0	1	NA	NA	NA	NA	0	0
Brain												
Macrophage aggregation, choroid plexus												
Minimal	0	0	2	2	0	0	NA	NA	NA	NA	2	2

-, no noteworthy findings; $AUC_{0-\tau}$, area under the plasma concentration-time curve during a time interval; BLQ, below the limit of quantitation; C_{max}, maximum plasma concentration; CREAT, Creatinine; F, female; M, male; NA, not applicable; NAG, N-Acetyl-beta-glucosaminidase.

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

b Number of observations (Number of animals affected).

c Incidence is presented.

Significantly different from control group (Group 1) value: A, $p \leq 0.05$; B, $p \leq 0.01$; C, $p \leq 0.001$ (T-test); D, $p \leq 0.05$; E, $p \leq 0.01$; F, $p \leq 0.001$ (Wilcoxon)

Group excluded from statistical analysis (N<3): Z

2.6.7.8 In Vitro 遺伝毒性試験

2.6.7.8.1 細菌を用いる復帰突然変異試験

<table>
<tr><td colspan="3">Report Title: Bacterial Mutation Test</td><td>Test Article: Setmelanotide (RM-493)</td></tr>
<tr><td colspan="2">Test for Induction of: Reverse mutations</td><td>No. of Independent Assays: 2</td><td>Study No. RM-493-TOX-008</td></tr>
<tr><td colspan="2">Strains: Salmonella typhimurium TA 98, TA100, TA1535, TA1537 and Escherichia coli WP2uvrA</td><td>No. of Replicate Cultures: 3</td><td>Location in eCTD: 4.2.3.3.1-1</td></tr>
<tr><td colspan="2">Metabolizing System: Phenobarbital/5,6-benzoflavone-induced rat liver S9 fraction</td><td>No. of Cells Analyzed/Culture: NA</td><td></td></tr>
<tr><td>Vehicles:</td><td>For Test Article: Water</td><td>For Positive Controls: Water for NaAz and 9AC, DMSO for 2NF, NQO, 2AA and BaP</td><td>GLP Compliance: Yes</td></tr>
<tr><td colspan="3">Treatment: Plate incorporation</td><td>Dates of Treatment:
December 7, 2010 (preliminary assay)
January 7, 2011 (confirmatory assay)</td></tr>
<tr><td colspan="4">Cytotoxic Effects: None</td></tr>
<tr><td colspan="4">Genotoxic Effects: None</td></tr>
</table>

Metabolic Activation	Treatment	Dose Level (µg/plate)	Revertants/plate (Mean ± SD)				
			TA1535	TA1537	TA98	TA100	WP2*uvrA*
Preliminary Assay							
Without Activation	Water	0	19 ± 3	19 ± 6	31 ± 6	168 ± 12	355[a] ± 15
	RM-493	27.5	21 ± 5	13 ± 4	29 ± 3	157 ± 15	349 ± 18
		87.1	19 ± 5	19 ± 7	37 ± 4	163 ± 17	348 ± 8
		278	15 ± 5	13 ± 1	28 ± 6	155 ± 13	341 ± 9
		885	15 ± 2	19 ± 4	33 ± 1	135 ± 14	338 ± 10
		2,782	19 ± 5	15 ± 4	29 ± 6	186 ± 7	366 ± 18
	NaAz	0.5	343 ± 4	NA	NA	584 ± 32	NA
	9AC	50	NA	332 ± 114	NA	NA	NA
	2NF	1	NA	NA	175 ± 15	NA	NA
	NQO	0.5	NA	NA	NA	NA	438 ± 26
With Activation	Water	0	21 ± 4	24 ± 7	56 ± 1	165 ± 25	373[a] ± 28
	RM-493	8.74	NA	17 ± 6	NA	NA	NA
		27.5	21 ± 4	17 ± 6	43 ± 13	186 ± 6	369 ± 26
		87.1	19 ± 3	19 ± 3	42 ± 11	190 ± 12	348 ± 22
		278	15 ± 3	19 ± 5	43 ± 9	160 ± 21	350 ± 3
		885	19 ±2	15 ± 3	43 ± 11	170 ± 18	351 ± 16
		2,782	20 ± 5	13[b] ± 5	46 ± 6	173 ± 8	354 ± 17
	2AA	5	460 ± 15	NA	NA	NA	NA
	2AA	15	NA	NA	NA	NA	495 ± 13
	BaP	5	NA	110 ± 15	439 ± 8	1083 ± 18	NA

Metabolic Activation	Treatment	Dose Level (µg/plate)	Revertants/plate (Mean ± SD)				
			TA1535	TA1537	TA98	TA100	WP2*uvrA*
Confirmatory Assay							
Without Activation	Water	0	16 ± 2	18 ± 1	35 ± 5	168 ± 7	53 ± 3
	RM-493	320	14 ± 6	11± 1	29 ± 2	169 ± 4	43 ± 5
		640	24 ± 2	15 ± 5	30 ± 2	180 ± 5	43 ± 8
		1,280	23 ± 3	15 ± 5	33 ± 15	174 ± 8	50 ± 2
		2,560	16 ± 9	14 ± 3	35 ± 9	168 ± 7	54 ± 16
		5,000	16 ± 5	10 ± 2	29 ± 5	162 ± 7	47 ± 7
	NaAz	0.5	373 ± 29	NA	NA	570 ± 26	NA
	9AC	50	NA	189 ± 59	NA	NA	NA
	2NF	1	NA	NA	197 ± 15	NA	NA
	NQO	0.5	NA	NA	NA	NA	145 ± 21
With Activation	Water	0	21 ± 2	22 ± 4	43 ± 6	188 ± 20	64 ± 7
	RM-493	80	NA	18 ± 2	NA	NA	NA
		160	NA	19 ± 2	NA	NA	NA
		320	18 ± 5	13 ± 3	45 ± 3	171 ± 11	59 ± 8
		640	20 ± 2	17 ± 3	41 ± 6	186 ± 5	72 ± 10
		1,280	22 ± 2	21 ± 2	47 ± 4	187 ± 15	64 ± 3
		2,560	20 ± 3	11[a] ± 3	43 ± 7	185 ± 2	68 ± 4
		5,000	13 ± 3	12[a] ± 3	48 ± 5	133 ± 19	70 ± 9
	2AA	5	501 ± 12	NA	NA	NA	NA
	2AA	15	NA	NA	NA	NA	244 ± 8
	BaP	5	NA	105 ± 12	452 ± 37	1004 ± 38	NA

Metabolic Activation	Treatment	Dose Level (µg/plate)	Revertants/plate (Mean ± SD)				
			TA1535	TA1537	TA98	TA100	WP2*uvrA*
Repeat Assay							
Without Activation	Water	0	NA	NA	NA	NA	53 ± 3
	RM-493	50	NA	NA	NA	NA	50 ± 6
		158	NA	NA	NA	NA	51 ± 8
		500	NA	NA	NA	NA	55 ± 3
		1,581	NA	NA	NA	NA	50 ± 4
		5,000	NA	NA	NA	NA	60 ± 6
	NQO	0.5	NA	NA	NA	NA	145 ± 21
With Activation	Water	0	NA	NA	NA	NA	64 ± 7
	RM-493	50	NA	NA	NA	NA	66 ± 6
		158	NA	NA	NA	NA	80 ± 3
		500	NA	NA	NA	NA	66 ± 4
		1,581	NA	NA	NA	NA	65 ± 7
		5,000	NA	NA	NA	NA	80 ± 1
	2AA	15	NA	NA	NA	NA	244 ± 8

2AA, 2-Aminoanthracene; 2NF, 2 Nitrofluorene; 9AC, 9-Aminoacridine; BaP, Benzo[a]pyrene; DMSO, Dimethyl sulfoxide; NA, Not applicable; NaAz, Sodium azide; NQO, 4-Nitroquinoline N-oxide; Water, Sterile Water for Irrigation, USP

a Over historical control range, this part of the assay was repeated due to invalid results.

b Toxic as indicated by low revertant colony counts compared to control vehicle treatment (fold response <0.6).

2.6.7.8.2 ヒト末梢血リンパ球を用いる染色体異常試験

Report Title: Chromosome Aberration Test			**Test Article:** Setmelanotide (RM-493)
Test for Induction of: Chromosome aberration		**No. of Independent Assays:** 1	**Study No.** RM-493-TOX-009
Cells: Primary Human Lymphocytes		**No. of Replicate Cultures:** 2	**Location in eCTD:** 4.2.3.3.1-2
Metabolizing System: Phenobarbital / 5,6-Benzoflavone-induced rat liver S9		**No. of Cells Analyzed/Culture:** 100	
Vehicle: Water	**Positive Controls:** Mitomycin C and Cyclophosphamide		**GLP Compliance:** Yes
Treatment: 4-hour treatment (initial) / 21-hour treatment (confirmatory) / 21-hour supplemental treatment (confirmatory)			**Dates of Treatment:** January 6, 2011 February 24, 2011
Cytotoxic Effects: Reduced mitotic indices (21-hour treatment)			
Genotoxic Effects: None.			

Initial Assay

Metabolic Activation	Test Article	Concentration (µg/mL)	Cytotoxicity[a] (% of Control)	Aberrant Cells (Mean %)[b]	Aberrations per Cell[b]	Total Polyploid Cells
Without activation	Water	0	100	0.5	0.005	1
	RM-493	1,280	105	0.0	0.000	0
		2,500	89	2.0	0.020	0
		5,000	91	1.5	0.015	1
	Mitomycin C	0.10	69	8.5**	0.085	0
With activation	Water	0	100	1.5	0.015	1
	RM-493	640	101	1.5	0.015	0
		1,280	106	1.0	0.010	0
		2,500	102	0.0	0.000	1
		5,000	84	0.6	0.000	2
	Cyclophosphamide	8.0	59	31.0**	0.280	1

Confirmatory Initial assay						
Metabolic Activation	**Test Article**	**Concentration (µg/mL)**	**Cytotoxicity[a] (% of Control)**	**Aberrant Cells (Mean %)[b]**	**Aberrations per Cell[b]**	**Total Polyploid Cells**
Without activation	Water	0	100	0.0	0.000	0
	RM-493	640	84	1.0	0.010	0
		1,280	66	2.5	0.025	0
		2,500	37	5.0**	0.050	0
	Mitomycin C	0.05	79	13.0**	0.120	1
Confirmatory Supplemental assay						
Without activation	Water	0	100	0.5	0.005	0
	RM-493	800	84	1.5	0.015	1
		966	58	0.5	0.005	0
		1,167	40	1.0	0.010	2
	Mitomycin C	0.05	79	11.0**	0.110	0

a Based on mitotic index.
b Does not include cells with only gaps.
Fisher's exact test * - $p<0.01$; ** - $p<0.001$

2.6.7.9 In Vivo 遺伝毒性試験

Report Title: RM-493 Mammalian Erythrocyte Micronucleus Test in Rat Bone Marrow		**Test Article:** Setmelanotide (RM-493)
Test for Induction of: Micronuclei in Bone Marrow Immature Erythrocytes	**Treatment Schedule:** 48 hours continuous subcutaneous infusion	**Study No.** RM-493-TOX-020 **Location in eCTD:** 4.2.3.3.2-1
Species/strain: Rat, Sprague Dawley® Hsd:SD® albino outbred	**Date of Treatment:** 24-26 January 2010	**GLP Compliance:** Yes
Age: 67 days old at treatment	**Sampling Time:** 24 hours following the end of infusion	
Cell Evaluated: Immature erythrocytes	**Method of administration:** Continuous subcutaneous infusion	
No. of Cells Analyzed/Animal: 2,000	**Vehicle for Test Article:** 0.9% Sodium Chloride Injection, USP	
Toxic/Cytotoxic Effects: Decreased activity, swelling (muzzle and/or lower jaw), and red skin (pinnae, hindpaws and/or forepaws) in males and/or females at 200 mg/kg/day. No significant decreases in the proportion of IE, indicating no bone marrow toxicity.		
Genotoxic Effects: None		

Test Article	**Dose (mg/kg/day)**	**No. of animals**	**Sampling time (hours)**	**Mean % IE/(IE+ME)**	**Incidence MIE**	
					Males	**Females**
Vehicle control	-	5M	24	40.4	2.2	NA
RM-493	50	5M	24	38.9	2.2	NA
RM-493	100	5M	24	38.2	2.2	NA
RM-493	200	5M+5F	24	34.5	1.8	1.8
Cyclophosphamide[a]	20	3M	24	32.5	22.7	NA

% IE/(IE+ME); Proportion of immature erythrocytes; ME, mature erythrocytes; MIE; Number of micronucleated cells observed per 2,000 immature erythrocytes examined; NA, not applicable.
a Administered once by oral gavage.

2.6.7.10 がん原性試験

2.6.7.10.1 がん原性試験：用量設定試験

					Test Article: Setmelanotide (RM-493)	
Species/ Strain	**Method of Administration**	**Duration of Dosing**	**Doses (mg/kg/day)**	**Gender and No. per Group**	**Noteworthy Findings**	**Study Number / Location in eCTD**
Mice/ (WT rasH2)	SC injection	7 days	0 (mPD); 10, 20, 40, 60, 80, 100 (1, 2, 4, 6, 8, 10 mg/mL in mPD; 10 mL/kg dose volume); 20, 40, 50 (4, 8, 10 mg/mL in mPD; 5 mL/kg dose volume)	5 males	≥20: Poorly tolerated at a dose volume of 10 mL/kg at concentrations ≥2 mg/mL and at a dose volume of 5 mL/kg at concentrations ≥4 mg/mL based on the incidence and severity of injection site reactions including eschar, edema, erythema, scab, and abrasion.	RM-493-TOX-040 / 4.2.3.4.2-1
Mice/ (WT rasH2)	SC injection	28 days	0 (saline), 0 (mPD), 10 (1, 2 mg/mL in mPD; 10, 5 mL/kg dose volume) 20 (4 mg/mL in mPD; 5 mL/kg dose volume)	Main: 10/sex TK: 12/sex controls; 48/sex treated	≥10: Dose-dependent injection site irritation including eschar, eschar exfoliation, edema, and erythema. At 10 mg/kg/day, irritation was more severe at a dose volume of 10 mL/kg (1 mg/mL) compared to 5 mL/kg (2 mg/mL); 20: Increased incidence of injection site thickening and swelling.	RM-493-TOX-041 / 4.2.3.4.2-2

mPD, mPEG-DSPE; SC, subcutaneous; TK, toxicokinetic; WT, wild-type.

2.6.7.10.2 rasH2-Tg マウスを用いた 6 ヵ月間皮下投与がん原性試験（1）

Report Title: A 6-month Carcinogenicity Study of RM-493 by Subcutaneous Injection in Tg.rasH2 Transgenic Mice		**Test Article:** Setmelanotide (RM-493)
Species/Strain: Transgenic rasH2 (CByB6F1- Tg(HRAS)2Jic Hemizygous) Mice Wild Type rasH2 (CByB6F1-Tg(HRAS)2Jic Wild Type) Mice	**Duration of Dosing:** RM-493: 26 weeks NMU: 1 day	**Study No.** RM-493-TOX-042 **Location in eCTD:** 4.2.3.4.2-3
Initial Age: 9 to 10 weeks old	**Duration of Postdose:** NA	**GLP Compliance:** Yes[a]
Date of First Dose: 20 Aug 2018	**Method of Administration:** Setmelanotide: Subcutaneous Injection NMU: Intraperitoneal Injection	
Vehicle/Formulation: Reference Item 1: 0.9% Sodium Chloride for Injection, USP; Reference Item 2: mPEG-DSPE Placebo; **Diluent for Test and Reference Items:** 5% Mannitol		
Special Features: Anti-therapeutic antibody evaluation; genotyping to determine the number of hemizygous Tg.rasH2 (TG) and wild-type (WT) animals per group following inadvertent randomization of TG and WT mice across all groups. Due to randomization of TG and WT mice into main study groups, selected data for TG + WT mice and TG mice only are shown.		
Conclusion: No evidence of a carcinogenic potential of setmelanotide in Tg.rasH2 mice		

Dose (mg/kg/day)	**0 (Saline)**		**0 (Placebo)**		**1**		**3**		**10**		**75 (NMU)[b]**	
Number of Animals/Sex/Group:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main (TG+WT):	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Bioanalytical:	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**NA**	**NA**
ADA:	**4**	**4**	**4**	**4**	**12**	**12**	**12**	**12**	**12**	**12**	**NA**	**NA**
Number of TG animals per Group (Main Study)	14	11	12	17	16	20	14	17	15	14	15	15
Plasma Concentrations (ng/mL)												
Day 1												
0 hour	BLQ	BLQ	BLQ	BLQ	0	0	0	0	0	0	NE	NE
1 hour	BLQ	BLQ	BLQ	BLQ	548	407	1,270	1,290	2,190	2,210	NE	NE
Day 182												
0 hour	BLQ	BLQ	BLQ	BLQ	0	0	0	0	0	0	NE	NE
1 hour	BLQ	BLQ	BLQ	BLQ	480	375	1,430	1,080	2,140	1,660	NE	NE
Anti-therapeutic Antibody	-	-	1[e]	-	-	-	-	1[f]	-	-	-	-

Results of Groups as Randomized (TG + WT)

Dose (mg/kg/day)	**0 (Saline)**		**0 (Placebo)**		**1**		**3**		**10**		**75 (NMU)[b]**	
Number of Animals/Sex/Group:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main (TG+WT):	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Main (TG):	**14**	**11**	**12**	**17**	**16**	**20**	**14**	**17**	**15**	**14**	**15**	**15**
Noteworthy Findings												
Died or Euthanized Moribund	1	0	0	0	0	1	3	1	1	0	19	20
Survival rate %	96	100	100	100	100	96	88	96	96	100	24[q]	20[q]
Body Weight	-	-	-	-	-	-	-	-	-	-	-	-
Food Consumption	-	-	-	-	-	-	-	-	-	-	-	-
Clinical Observations[c]												
Abdominal distension	0	0	0	0	0	0	0	0	0	0	9 (5)	5 (3)
Abnormal firm internal structure	0	0	0	0	0	0	0	0	0	0	7 (5)	3 (3)
Abnormal gait	0	0	0	0	0	0	0	0	0	0	1 (1)	5 (4)
Activity decreased	0	0	0	0	0	1 (1)	1 (1)	0	0	0	10 (10)	7 (7)
Backbone prominent	4 (1)	4 (4)	3 (3)	3 (2)	1 (1)	1 (1)	6 (3)	7 (7)	3 (2)	3 (3)	35 (18)	20 (13)
Breathing												
Abnormal sounds	0	0	0	0	0	0	0	0	0	0	0	1 (1)
Deep	0	0	0	0	0	0	0	0	0	0	2 (2)	2 (2)
Labored	0	0	0	0	0	1 (1)	0	1 (1)	0	0	9 (9)	8 (8)
Shallow	1 (1)	0	0	0	0	0	1 (1)	0	0	0	14 (11)	12 (10)
Cold to touch	0	0	0	0	0	0	0	0	1 (1)	0	8 (8)	7 (7)
Dehydrated suspected	3 (1)	0	0	0	0	0	5 (3)	0	1 (1)	0	30 (16)	17 (13)
Fur, staining, black	2 (1)	0	0	0	39 (15)	12 (7)	86 (23)	95 (24)	100 (25)	108 (25)	0	0
Hunched posture	0	0	0	0	0	0	0	1 (1)	3 (2)	0	10 (9)	9 (9)

Dose (mg/kg/day)	0 (Saline)		0 (Placebo)		1		3		10		75 (NMU)[b]	
Number of Animals/Sex/Group:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main (TG+WT):	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Main (TG):	**14**	**11**	**12**	**17**	**16**	**20**	**14**	**17**	**15**	**14**	**15**	**15**
Injection site												
Fur, thin cover	4 (1)	1 (1)	32 (1)	0	9 (4)	2 (1)	32 (5)	11 (4)	4 (2)	51 (8)	28 (3)	25 (5)
Skin, pale	0	7 (2)	0	0	1 (1)	0	4 (2)	20 (2)	71 (4)	27 (3)	0	0
Skin, scab	25 (10)	5 (5)	32 (14)	7 (6)	19 (17)	7 (7)	33 (18)	15 (8)	98 (18)	41 (15)	36 (14)	27 (15)
Skin thickening	14 (3)	1,493 (18)	232 (15)	1,941 (20)	152 (10)	886 (16)	301 (19)	1,116 (18)	956 (20)	2,646 (25)	0	8 (4)
Skin, white	26 (5)	621 (15)	14 (3)	584 (16)	61 (2)	378 (11)	110 (4)	397 (14)	334 (11)	855 (19)	0	1 (1)
Swollen firm	0	0	1 (1)	1 (1)	0	0	1 (1)	0	1 (1)	3 (2)	15 (1)	3 (3)
Swollen soft	1 (1)	7 (2)	3 (2)	17 (8)	0	0	1 (1)	0	17 (5)	25 (2)	0	0
Muscle tone decreased	0	0	0	0	0	1 (1)	0	0	0	0	1 (1)	1 (1)
Respiratory rate												
increased	1 (1)	0	0	0	0	1 (1)	1 (1)	0	0	0	6 (4)	4 (4)
irregular	0	0	0	0	0	0	0	0	0	0	3 (3)	1 (1)
Thin	4 (1)	0	0	4 (2)	0	2 (2)	5 (3)	3 (3)	1 (1)	1 (1)	30 (17)	22 (14)
Tremors	0	0	0	0	0	0	0	0	0	0	1 (1)	0
Weak	1 (1)	0	0	0	0	1 (1)	0	0	0	0	1 (1)	1 (1)
Palpable Mass												
Number	0	0	0	0	0	0	0	0	0	1	2	7
%	0	0	0	0	0	0	0	0	0	4	8	28

Dose (mg/kg/day)	0 (Saline)		0 (Placebo)		1		3		10		75 (NMU)[b]	
Number of Animals/Sex/Group:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main (TG+WT):	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Main (TG):	**14**	**11**	**12**	**17**	**16**	**20**	**14**	**17**	**15**	**14**	**15**	**15**
Gross Pathology[d]												
Number of animals examined	25	25	25	25	25	25	25	25	25	25	25	25
Liver												
Discoloration; pale	0	1	1	0	0	0	1	0	0	0	9	5
Enlargement	0	0	1	0	0	0	0	0	0	0	7	4
Lung												
Discoloration; pale	1	0	0	0	0	0	2	1	0	0	3	3
Nodule	0	0	1	1	1	1	2	0	2	2	4	5
Skin												
Nodule	0	0	0	0	0	0	0	0	0	0	2	3
Mass	0	0	0	0	0	0	0	0	0	0	1	6
Spleen												
Enlargement	0	1	1	2	0	4	0	5	0	6	17	15
Discoloration; pale	1	0	0	0	0	0	0	1	0	0	7	5
Stomach												
Focus; raised	0	0	0	0	0	0	1	0	2	0	2	2
Nodule	0	0	0	0	0	0	0	0	0	0	8	8
Mass	0	0	0	1	0	0	0	0	1	0	3	5
Thymus												
Mass	0	0	0	0	0	0	1	0	0	0	13	11

Dose (mg/kg/day)	0 (Saline)		0 (Placebo)		1		3		10		75 (NMU)[b]	
Number of Animals/Sex/Group:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main (TG+WT):	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Main (TG):	**14**	**11**	**12**	**17**	**16**	**20**	**14**	**17**	**15**	**14**	**15**	**15**
Histopathology[d]												
Neoplastic findings												
Number of animals examined	25	25	25	25	25	25	25	25	25	25	25	25
Hemolymphoreticular tissue												
Lymphoma, malignant	0	0	0	0	1	0	0	0	0	0	19[g]	21[g]
Lung												
Bronchioloalveolar adenoma	1	0	0	1	1	2	2	0	0	1	7[k]	6[h]
Bronchioloalveolar carcinoma	1	0	0	0	0	0	2	0	0	0	0	0
Skin												
Papilloma	0	0	0	0	0	0	0	0	0	0	2	6[i]
Squamous cell carcinoma	0	0	0	0	0	0	0	0	0	0	1	4
Stomach												
Papilloma	0	0	0	0	0	0	1	0	0	0	12[g]	10[j]
Squamous cell carcinoma	0	0	0	0	0	0	0	0	0	0	6[l]	2
Non-Neoplastic Findings												
Site, injection	25	25	25	25	25	25	25	25	25	25	NA	NA
Infiltration, vacuolated macrophages	3	0	25	25	25	25	25	24	25	25	NA	NA
Minimal	3	0	4	6	25	25	11	16	1	0	NA	NA
Mild	0	0	21	19	0	0	14	8	18	20	NA	NA
Moderate	0	0	0	0	0	0	0	0	6	5	NA	NA
Infiltration, mixed cell	20	22	24	25	23	24	21	25	24	25	NA	NA
Minimal	19	14	15	20	21	21	16	20	10	12	NA	NA
Mild	1	8	8	5	2	3	5	5	13	11	NA	NA
Moderate	0	0	1	0	0	0	0	0	1	2	NA	NA

Results from Hemizygous Tg.rasH2 (TG) Mice Only

Dose (mg/kg/day)	0 (Saline)		0 (Placebo)		1		3		10		75 (NMU)[b]	
Number of Animals/Sex/Group:	M	F	M	F	M	F	M	F	M	F	M	F
Main (TG):	14	11	12	17	16	20	14	17	15	14	15	15
Noteworthy Findings												
Died or Euthanized Moribund	1	0	0	0	0	1	3	1	1	0	14	14
Survival rate %	93	100	100	100	100	95	79	94	96	100	7[q]	7[q]
Histopathology[d]												
Neoplastic findings												
Number of animals examined	14	11	12	17	16	20	14	17	15	14	15	15
Hemolymphoreticular tissue												
Lymphoma, malignant	0	0	0	0	1	0	0	0	0	0	13[g]	12[g]
Lung												
Bronchioloalveolar adenoma	1	0	0	1	1	2	1	0	0	1	4	3
Bronchioloalveolar carcinoma	1	0	0	0	0	0	2	0	0	0	0	0
Skin												
Papilloma	0	0	0	0	0	0	0	0	0	0	2	6[m]
Squamous cell carcinoma	0	0	0	0	0	0	0	0	0	0	0	4[n]
Stomach												
Papilloma	0	0	0	0	0	0	1	0	0	0	11[g]	9[o]
Squamous cell carcinoma	0	0	0	0	0	0	0	0	0	0	6[p]	2

-, no noteworthy findings; ADA, anti-drug antibodies; BLQ, below the limit of quantitation; F, female; M, male; NA, not applicable; NE, not evaluated; NMU, N-Nitrosomethylurea; TG, hemizygous Tg.rasH2 mice; WT, wild-type mice.

a With the exception of test article characterization and stability (Good Manufacturing Practice), genotyping analysis, and serum sample ADA analysis which exceeded the established long term serum sample stability by up to 534 days. All reported ADA values are considered valid, supported by literature references indicating stability of antibodies at 20°C or lower for at least 4.6 years.

b Single dose of NMU administered by intraperitoneal injection on Day 1.

c Number of occurrences (Number of animals affected).

d Incidence is presented.

e Positive immunodepletion result in 1 out of 4 vehicle control males evaluated for ADA

f Positive immunodepletion result in 1 out of 12 low dose females evaluated for ADA

g Comparison between Group 2 and Group 6 <0.0001, using a One-Sided Fisher's Exact test, revealed statistical significance, p-value 0.05

h Comparison between Group 2 and Group 6 <0.0491, using a One-Sided Fisher's Exact test, revealed statistical significance, p-value 0.05

i Comparison between Group 2 and Group 6 <0.0127, using a One-Sided Fisher's Exact test, revealed statistical significance, p-value 0.05
j Comparison between Group 2 and Group 6 <0.0003, using a One-Sided Fisher's Exact test, revealed statistical significance, p-value 0.05
k Comparison between Group 2 and Group 6 <0.0048, using a One-Sided Fisher's Exact test, revealed statistical significance, p-value 0.05
l Comparison between Group 2 and Group 6 <0.0111, using a One-Sided Fisher's Exact test, revealed statistical significance, p-value 0.05
m Comparison between Group 2 and Group 6 <0.0055, using a One-Sided Fisher's Exact test, revealed statistical significance, p-value 0.05
n Comparison between Group 2 and Group 6 <0.0360, using a One-Sided Fisher's Exact test, revealed statistical significance, p-value 0.05
o Comparison between Group 2 and Group 6 <0.0002, using a One-Sided Fisher's Exact test, revealed statistical significance, p-value 0.05
p Comparison between Group 2 and Group 6 <0.0169, using a One-Sided Fisher's Exact test, revealed statistical significance, p-value 0.05
q Comparison between Group 2 and Group 6, using a Peto's Two-Sided test, revealed statistical significance, p-value <0.0001

2.6.7.10.3 rasH2-Tg マウスを用いた 6 ヵ月間皮下投与がん原性試験（2）

Report Title: A 6-month Carcinogenicity Study of RM-493 by Subcutaneous Injection in Tg.rasH2 Transgenic Mice		**Test Article:** Setmelanotide (RM-493)
Species/Strain: Transgenic rasH2 (CByB6F1-Tg(HRAS)2Jic Hemizygous) Mice	**Duration of Dosing:** RM-493: 26 weeks NMU: 1 day	**Study No.** RM-493-TOX-044 **Location in eCTD:** 4.2.3.4.2-4
Initial Age: 8 weeks old	**Duration of Postdose:** NA	
Date of First Dose: 22 July, 2019	**Method of Administration:** Setmelanotide: Subcutaneous Injection NMU: Intraperitoneal Injection	**GLP Compliance:** Yes[a]
Vehicle/Formulation: Reference Item 1: 0.9% Sodium Chloride for Injection, USP; Reference Item 2: mPEG-DSPE Placebo; Diluent for Test Item and Reference Item 2: 5% Mannitol		
Special Features: All animals in this study were transgenic mice (no wild type mice on study); Anti-drug antibody evaluation; Malfunction of water supply system resulting in cage flooding and death of 10 high-dose males in Week 18.		
Conclusion: No evidence of carcinogenic potential or anti-RM-493 antibodies in Tg.rasH2 mice at doses up to 10 mg/kg/day.		

Dose (mg/kg/day)	**0 (Saline)**		**0 (Placebo)**		**1**		**3**		**10**		**75 (NMU)[b]**	
Number of Animals/Sex/Group:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	25	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Plasma Concentrations (ng/mL)												
Day 182												
1 hour	BLQ	BLQ	BLQ	BLQ	462		1,130		1,950		NE	NE
24 hour	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NE	NE
Anti-therapeutic Antibodies	-	-	-	-	-	-	-	-	-	-	-	-
Noteworthy Findings												
Died or Euthanized Moribund (excluding accidental deaths)	3	1	1	0	0	2	2	1	2	0	22	25
Died or Euthanized Moribund (including accidental deaths)	3	1	1	0	0	2	2	1	12[i]	1	23	25

Report Title: A 6-month Carcinogenicity Study of RM-493 by Subcutaneous Injection in Tg.rasH2 Transgenic Mice									**Test Article:** Setmelanotide (RM-493)			
Dose (mg/kg/day)	**0 (Saline)**		**0 (Placebo)**		**1**		**3**		**10**		**75 (NMU)[b]**	
Number of Animals/Sex/Group:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	25	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Survival rate % (excluding accidental deaths)	88	96	96	100	100	92	92	96	87	100	8[e]	0[e]
Survival rate % (including accidental deaths)	88	96	96	100	100	92	92	96	52[i]	96	8[e]	0[e]
Body Weight	-	-	-	-	-	-	-	-	-	-	-	-
Food Consumption	-	-	-	-	-	-	-	-	-	-	-	-
Clinical Observations[c]												
Abdominal distension	0	0	3 (2)	0	0	0	0	0	0	4 (1)	13 (4)	13 (8)
Abnormal gait	0	0	0	0	0	2 (2)	0	0	0	0	8 (6)	3 (3)
Activity decreased	0	1 (1)	1 (1)	0	0	1 (1)	0	0	0	0	19 (18)	15 (14)
Backbone prominent	3 (1)	10 (8)	2 (1)	4 (2)	0	4 (2)	1 (1)	2 (1)	2 (1)	0	29 (17)	19 (13)
Breathing												
Abnormal sounds	0	0	0	0	0	0	0	0	0	0	1 (1)	0
Deep	0	0	0	0	0	0	0	0	0	0	2 (1)	4 (4)
Labored	0	1 (1)	0	0	0	0	0	0	0	0	6 (6)	11 (10)
Shallow	2 (1)	0	1 (1)	0	0	1 (1)	0	0	1 (1)	0	32 (17)	23 (11)
Cold to touch	0	1 (1)	1 (1)	0	0	0	0	0	0	0	6 (6)	12 (12)
Dehydrated suspected	5 (3)	4 (3)	1 (1)	0	2 (2)	6 (4)	1 (1)	0	2 (2)	0	51 (23)	32 (17)
Fur erected	0	1 (1)	0	0	0	1 (1)	0	0	1 (1)	0	52 (22)	31 (15)
Hunched posture	1 (1)	4 (4)	0	1 (1)	2 (2)	2 (2)	0	1 (1)	3 (3)	1 (1)	33 (15)	31 (17)
Injection site 1												
Skin, scab	10 (7)	2 (2)	20 (9)	8 (4)	23 (10)	10 (6)	16 (10)	3 (2)	73 (16)	14 (6)	0	0
Skin thickening	99 (14)	129 (17)	695 (24)	806 (25)	291 (23)	596 (24)	457 (24)	804 (25)	788 (23)	1,079 (24)	0	0
Skin, discolored	5 (4)	34 (5)	124 (14)	373 (18)	36 (12)	166 (14)	50 (12)	372 (20)	120 (17)	435 (19)	1 (1)	0
Injection site 2												
Skin, scab	15 (5)	2 (2)	11 (7)	3 (1)	34 (11)	8 (5)	27 (11)	9 (7)	89 (21)	20 (12)	0	0

Report Title: A 6-month Carcinogenicity Study of RM-493 by Subcutaneous Injection in Tg.rasH2 Transgenic Mice									**Test Article:** Setmelanotide (RM-493)			
Dose (mg/kg/day)	**0 (Saline)**		**0 (Placebo)**		**1**		**3**		**10**		**75 (NMU)[b]**	
Number of Animals/Sex/Group:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	25	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Skin thickening	77 (13)	103 (14)	646 (24)	792 (25)	270 (25)	542 (24)	403 (24)	797 (25)	729 (23)	1,076 (24)	0	0
Skin, discolored	15 (7)	28 (5)	104 (13)	339 (17)	43 (15)	140 (12)	38 (13)	353 (20)	133 (17)	433 (21)	0	0
Injection site 3												
Skin, scab	10 (5)	3 (3)	13 (7)	2 (4)	21 (8)	0	21 (8)	4 (4)	52 (17)	21 (7)	0	0
Skin thickening	48 (14)	102 (8)	546 (24)	511 (25)	198 (24)	432 (22)	382 (24)	699 (25)	706 (23)	1,049 (25)	0	0
Skin, discolored	15 (8)	56 (5)	127 (13)	310 (16)	34 (9)	184 (14)	50 (17)	384 (19)	185 (16)	529 (21)	0	0
Injection site 4												
Skin, scab	7 (4)	0	9 (6)	4 (2)	16 (5)	4 (2)	17 (8)	12 (7)	40 (14)	37 (10)	0	0
Skin thickening	65 (16)	90 (8)	577 (24)	527 (25)	203 (24)	438 (21)	408 (24)	723 (25)	751 (23)	1,068 (24)	0	0
Skin, discolored	14 (8)	46 (4)	150 (14)	286 (14)	40 (12)	182 (12)	61 (16)	402 (18)	160 (14)	516 (22)	0	0
Muscle tone, generalized	0	0	0	0	0	0	1 (1)	0	0	0	9 (8)	5 (5)
Respiratory rate, abnormal	3 (1)	0	0	0	0	0	0	1 (1)	0	0	12 (9)	15 (12)
Skin pallor	0	1 (1)	0	0	0	0	0	0	0	0	4 (4)	6 (6)
Thin	3 (1)	17 (9)	2 (1)	4 (3)	0	11 (5)	1 (1)	7 (4)	2 (1)	0	26 (18)	34 (17)
Tremors	0	0	0	0	0	0	0	0	0	0	2 (2)	2 (1)
Weak	0	0	0	0	0	0	0	0	0	0	3 (3)	5 (5)
Palpable Masses												
Number	1	0	1	0	1	1	1	1	0	1	17	12
%	4	0	4	0	4	4	4	4	0	4	68	48
Gross Pathology[d]												
Liver												
Discoloration; pale	1	1	1	1	0	1	1	1	0	0	8	9
Enlargement	0	0	0	0	0	0	0	0	0	0	6	7
Spleen												
Discoloration; pale	0	1	0	0	0	0	0	0	0	0	9	10

Report Title: A 6-month Carcinogenicity Study of RM-493 by Subcutaneous Injection in Tg.rasH2 Transgenic Mice									**Test Article:** Setmelanotide (RM-493)			
Dose (mg/kg/day)	**0 (Saline)**		**0 (Placebo)**		**1**		**3**		**10**		**75 (NMU)[b]**	
Number of Animals/Sex/Group:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	25	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Enlargement	1	0	0	0	0	4	2	1	0	1	20	18
Kidney												
Enlargement	0	0	1	0	0	0	0	0	0	0	7	0
Lymph node, mandibular												
Enlargement	0	1	0	0	0	0	0	0	0	0	9	11
Lymph node, mesenteric												
Enlargement	0	0	0	0	0	0	0	0	0	0	12	12
Thymus												
Mass	0	0	0	0	0	0	0	0	0	0	9	6
Body cavity, thoracic												
Fluid accumulation, dark	0	0	0	0	0	0	1	1	1	0	1	8
Lung												
Nodule	0	0	1	0	1	0	1	0	0	3	4	3
Mass	1	1	1	0	0	0	0	0	1	0	3	0
Skin												
Nodule	0	0	0	0	0	0	0	0	0	0	8	7
Mass	1	0	0	0	1	1	1	0	0	0	10	4
Stomach												
Focus; raised	0	0	1	0	0	1	0	1	2	1	4	8
Nodule	3	1	0	0	0	0	0	0	0	0	22	19
Mass	1	1	0	2	0	2	0	0	0	2	10	3

Report Title: A 6-month Carcinogenicity Study of RM-493 by Subcutaneous Injection in Tg.rasH2 Transgenic Mice									**Test Article:** Setmelanotide (RM-493)			
Dose (mg/kg/day)	**0 (Saline)**		**0 (Placebo)**		**1**		**3**		**10**		**75 (NMU)[b]**	
Number of Animals/Sex/Group:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	25	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Histopathology												
Neoplastic Changes[d]												
Hemolymphoreticular tissue												
Lymphoma, malignant	0	0	0	0	0	0	0	0	0	0	23[f]	21[f]
Skin												
Papilloma; squamous cell	0	0	0	0	0	1	1	0	0	0	12[f]	6[g]
Squamous cell carcinoma	1	0	0	0	0	0	0	0	0	1	3	0
Stomach												
Papilloma; squamous cell	3	0	0	0	0	0	0	0	0	0	23[f]	22[f]
Squamous cell carcinoma	0	0	1	0	0	0	0	0	0	0	5	3
Spleen												
Hemangiosarcoma	3	0	1	1	1	0	1	1	1	1	0	3
Lung												
Bronchioloalveolar adenoma	1	0	1	1	1	0	1	0	0	2	6[h]	4
Bronchioloalveolar carcinoma	1	1	1	0	0	0	0	0	1	1	1	0
Non-Neoplastic Findings[d]												
Site, injection												
Crust; serocellular												
Minimal	4	2	1	1	4	4	9	5	3	5	NA	NA
Mild	2	0	2	0	2	1	1	1	5	2	NA	NA
Moderate	0	0	0	0	1	0	1	0	0	0	NA	NA
Infiltration, mixed cell												
Minimal	10	10	15	12	15	14	9	14	4	9	NA	NA
Mild	12	15	9	13	10	7	15	8	20	11	NA	NA
Moderate	0	0	0	0	0	0	0	0	0	5	NA	NA

Report Title: A 6-month Carcinogenicity Study of RM-493 by Subcutaneous Injection in Tg.rasH2 Transgenic Mice									**Test Article:** Setmelanotide (RM-493)			
Dose (mg/kg/day)	**0 (Saline)**		**0 (Placebo)**		**1**		**3**		**10**		**75 (NMU)[b]**	
Number of Animals/Sex/Group:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	25	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Infiltration, vacuolated macrophages												
Minimal	0	0	0	3	24	22	10	14	1	0	NA	NA
Mild	0	0	13	20	1	3	15	9	4	3	NA	NA
Moderate	0	0	12	2	0	0	0	1	16	20	NA	NA
Marked	0	0	0	0	0	0	0	0	4	2	NA	NA

-, no noteworthy findings; BLQ, below the limit of quantitation; F, female; M, male; NMU, N-Nitrosomethylurea; NA, not applicable; NE, not evaluated.

a With the exception of test article characterization and stability (GMP).

b Single dose of NMU administered by intraperitoneal injection on Day 1.

c Number of occurrences (Number of animals affected)

d Incidence is presented.

e Comparison between Group 2 and Group 6, using a Peto's test, revealed statistical significance, p value <0.0001

f Comparison between Group 2 and Group 6 <0.0001, using a One-Sided Fisher's Exact test, revealed statistical significance, p value 0.05

g Comparison between Group 2 and Group 6 = 0.0111, using a One-Sided Fisher's Exact test, revealed statistical significance, p value 0.05

h Comparison between Group 2 and Group 6 = 0.0491, using a One-Sided Fisher's Exact test, revealed statistical significance, p value 0.05

i Ten males died from drowning due to malfunction of water supply system.

2.6.7.10.4 rasH2-Tg マウスを用いた 6 ヵ月間皮下投与がん原性試験（3）

Report Title: A 6-month Carcinogenicity Study of RM-493 by Subcutaneous Injection in Tg.rasH2 Transgenic Mice		**Test Article:** Setmelanotide (RM-493)
Species/Strain: Transgenic rasH2 (CByB6F1-Tg(HRAS)2Jic Hemizygous) Mice	**Duration of Dosing:** RM-493: 26 weeks NMU: 1 day	**Study No.** RM-493-TOX-045
Initial Age: 8 weeks old	**Duration of Postdose:** NA	**Location in eCTD:** 4.2.3.4.2-5
Date of First Dose: 21 January, 2020	**Method of Administration:** Setmelanotide: Subcutaneous Injection NMU: Intraperitoneal Injection	**GLP Compliance:** Yes[a]
Vehicle/Formulation: Reference Item 1: 0.9% Sodium Chloride for Injection, USP; Reference Item 2: mPEG-DSPE Placebo; Diluent for Test Item and Reference Item 2: 5% Mannitol		
Special Features: All animals in the study were transgenic mice (no wild type mice on study); Due to a change in bioanalytical laboratories, anti-drug antibody evaluation is pending and will be submitted in an amended study report, as a post-authorization commitment.		
Conclusion: No evidence of carcinogenic potential in Tg.rasH2 mice at doses up to 10 mg/kg/day (0.2, 0.6, and 2 mg/mL at 5 mL/kg).		

Dose (mg/kg/day)	**0 (Saline)**		**0 (Placebo)**		**1**		**3**		**10**		**75 (NMU)[b]**	
Number of Animals/Sex/Group:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	25	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Plasma Concentrations (ng/mL)												
Day 182												
1 hour	BLQ	BLQ	BLQ	BLQ	458	381	1,160	1,190	2,150	3,010	NE	NE
24 hour	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NE	NE
Anti-Drug Antibodies	-	-	-	-	-	-	-	-	-	-	NA	NA
Noteworthy Findings												
Died or Euthanized Moribund	0	0	0	0	1	0	0	2	1	0	24	23
Survival rate %	100	100	100	100	96	100	100	92	96	100	4[e]	8[e]
Body Weight	-	-	-	-	-	-	-	-	-	-	-	-
Food Consumption	-	-	-	-	-	-	-	-	-	-	-	-

Report Title: A 6-month Carcinogenicity Study of RM-493 by Subcutaneous Injection in Tg.rasH2 Transgenic Mice									**Test Article:** Setmelanotide (RM-493)			
Dose (mg/kg/day)	**0 (Saline)**		**0 (Placebo)**		**1**		**3**		**10**		**75 (NMU)[b]**	
Number of Animals/Sex/Group:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	25	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Clinical Observations[c]												
Abdominal distension	0	0	0	0	0	0	0	0	0	0	4 (2)	13 (3)
Activity decreased	0	0	0	0	0	0	0	1 (1)	1 (1)	0	14 (13)	8 (7)
Breathing labored	0	0	0	0	0	0	0	1 (1)	1 (1)	0	10 (9)	9 (7)
Respiratory rate abnormal	0	0	0	0	1 (1)	0	0	1 (1)	0	0	18 (12)	7 (5)
Cold to touch	0	0	0	0	0	0	0	0	0	0	3 (3)	5 (4)
Dehydration	0	0	0	0	0	0	0	2 (2)	2 (1)	0	55 (23)	36 (18)
Hunched posture	0	0	0	1 (1)	0	1 (1)	0	1 (1)	0	1(1)	25 (10)	6 (4)
Weak	0	0	0	0	0	0	0	0	1 (1)	0	6 (5)	5 (3)
Palpable Masses												
Number of mice	0	0	0	0	0	0	1	0	0	0	2	3
%	0	0	0	0	0	0	4	0	0	0	8	12
Gross Pathology[d]												
Liver												
Discoloration; pale	0	0	0	0	0	1	0	0	0	0	3	2
Enlargement	0	0	0	0	0	0	0	0	0	0	3	2
Spleen												
Discoloration; pale	0	0	0	0	0	0	0	0	0	0	5	0
Enlargement	0	0	1	1	0	0	1	0	0	0	16	15
Kidney												
Enlargement	0	0	0	0	0	0	0	0	0	0	5	4
Lymph node, mandibular												
Enlargement	0	0	0	0	1	0	0	0	1	0	10	8
Lymph node, mesenteric												
Enlargement	0	0	0	0	0	0	0	0	0	0	11	9

Report Title: A 6-month Carcinogenicity Study of RM-493 by Subcutaneous Injection in Tg.rasH2 Transgenic Mice									**Test Article:** Setmelanotide (RM-493)			
Dose (mg/kg/day)	**0 (Saline)**		**0 (Placebo)**		**1**		**3**		**10**		**75 (NMU)[b]**	
Number of Animals/Sex/Group:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	25	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Thymus												
Mass	0	0	0	0	0	0	0	0	0	0	14	17
Lung												
Nodule	2	0	1	0	2	1	1	0	1	2	2	2
Mass	0	0	0	0	0	1	0	0	1	2	0	0
Skin												
Nodule	1	0	0	0	0	0	0	0	1	0	3	1
Mass	0	0	0	0	2	0	1	0	0	0	4	6
Stomach												
Focus; raised	0	0	0	0	0	0	0	0	0	0	12	12
Nodule	0	0	0	0	0	0	0	0	1	0	7	4
Mass	0	1	0	3	0	1	0	2	0	1	6	3
Histopathology												
Neoplastic Changes[d]												
Hemolymphoreticular tissue												
Lymphoma, malignant	0	0	0	0	0	0	0	0	0	0	20[f]	20[f]
Skin												
Papilloma; squamous cell	0	0	0	0	2	0	0	0	0	0	7[f]	1
Squamous cell carcinoma	0	0	0	0	0	0	0	0	0	0	0	3
Stomach												
Papilloma; squamous cell	0	0	0	0	0	0	0	0	0	0	11[f]	10[f]
Squamous cell carcinoma	0	0	0	0	0	0	0	0	0	0	6[f]	6[f]
Spleen												
Hemangiosarcoma	0	2	2	3	1	1	3	1	1	2	1	2

Report Title: A 6-month Carcinogenicity Study of RM-493 by Subcutaneous Injection in Tg.rasH2 Transgenic Mice									**Test Article:** Setmelanotide (RM-493)			
Dose (mg/kg/day)	**0 (Saline)**		**0 (Placebo)**		**1**		**3**		**10**		**75 (NMU)[b]**	
Number of Animals/Sex/Group:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	25	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Lung												
Bronchioloalveolar adenoma	2	1	1	1	2	1	0	1	2	1	1	5
Bronchioloalveolar carcinoma	1	0	0	0	0	1	0	0	2	2	1	0
Uterus												
Polyp, endometrial	NA	0	NA	0	NA	0	NA	0	NA	1	NA	6[f]
Non-Neoplastic Findings[d]												
Site, injection												
Inflammation, mixed cell												
Minimal	23	18	7	11	22	24	18	16	1	1	NA	NA
Mild	1	6	18	14	2	1	7	8	19	17	NA	NA
Moderate	0	0	0	0	0	0	0	0	4	7	NA	NA
Vacuolated macrophages												
Minimal	-	-	5	0	18	11	18	9	5	0	NA	NA
Mild	-	-	20	25	6	14	7	15	19	25	NA	NA
Acanthosis												
Minimal	-	-	4	1	-	-	2	-	7	0	NA	NA
Mild	-	-	0	0	-	-	1	-	3	1	NA	NA
Moderate	-	-	0	0	-	-	0	-	0	1	NA	NA

-, no noteworthy findings; BLQ, below the limit of quantitation; F, female; M, male; NMU, N-Nitrosomethylurea; NA, not applicable; NE, not evaluated.

a With the exception of test article characterization and stability (GMP).

b Single dose of NMU administered by intraperitoneal injection on Day 1.

c Number of occurrences (Number of animals affected)

d Incidence is presented.

e Significantly different from Group 2, $p < 0.0001$, Peto's test

f Significantly different from Group 2 $p \leq 0,05$ pairwise comparison test

2.6.7.11 生殖発生毒性試験：重要な試験以外の試験

						Test Article: Setmelanotide (RM-493)	
Type of Study	**Species/ Strain**	**Method of Administration**	**Dosing Period**	**Doses (mg/kg/day)**	**No. per Group**	**Noteworthy Findings**	**Study Number / Location in eCTD**
Dose range-finding EFD	NZW rabbit	Subcutaneous injection	GD 7-13 GD 13-19	0 (mPEG-DSPE), 0.05, 0.5, 3, 10, 20	Control: 5 females[a] RM-493: 6 females[b]	≥0.05: Marked reductions in food consumption ≥0.5: Decreased body weight; abortion and dose-related post-implantation less related to total resorption in GD 13-19 subgroup. ≥3: Dose-related post-implantation loss associated with total resorption in GD 7 to 13 subgroup	RM-493-TOX-030/ 4.2.3.5.2-1

EFD, embryo-fetal development; GD, gestation day; NZW, New Zealand white.

a All animals dosed from GD 7 to 19.

b Three animals/group were dosed from GD 7 to 13; the other 3 animals/group were dosed from GD 13 to 19.

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

Setmelanotide/mPEG-DSPE の雄受胎能及び生殖能に対する影響は「ラットを用いた 26 週間皮下投与毒性試験及び 4 週間回復性試験」（2.6.7.7.1 項）で評価し、雌受胎能及び初期胚発生に対する影響は「雌ラットを用いた受胎能及び胚・胎児発生に関する試験」（2.6.7.13.1 項）で評価した。

2.6.7.13 生殖発生毒性試験：胚・胎児発生に関する試験

2.6.7.13.1 雌ラットを用いた受胎能及び胚・胎児発生に関する試験

Report Title: A Fertility and Embryo-fetal Development Study of RM-493 by Subcutaneous Injection in Female Rats		**Test Article:** Setmelanotide **(**RM-493)
Design similar to ICH S5(R2)? Yes	**Duration of Dosing:** 14 days prior to cohabitation (SD 1) to GD 17	**Study No.** RM-493-TOX-029
Species/Strain: Sprague Dawley rats	**Day of Mating:** GD 0	**Location in eCTD:** 4.2.3.5.1-1
	Day of C-Section: GD 21	
Initial Age:7 to 8 weeks old	**Method of Administration:** Subcutaneous injection	**GLP Compliance**: Yes
Date of First Dose: 26 Apr 2016	**Vehicle/Formulation:** mPEG-DSPE	

Special Features: Dosing period covers female fertility and embryonic development; anti-drug antibody assessment; Clinical setmelanotide/mPEG-DSPE and mPEG-DSPE vehicle formulations diluted 10-fold with 5% Mannitol in sterile water for injection (USP)

No Observed Adverse Effect Level:
F_0 Females (including fertility): 5.0 mg/kg/day
F_1 Litters: 5.0 mg/kg/day

Daily Dose (mg/kg)	**0 (Vehicle)**	**0 (Saline)**	**0.5**	**3.0**	**5.0**
Number of Females: Main:	**22**	**22**	**22**	**22**	**22**
Toxicokinetics:	**3**	**3**	**9**	**9**	**9**
F_0 Females:					
Toxicokinetics:					
SD 1					
C_{max} (ng/mL)	NA	NA	130	470	445
$AUC_{0-\tau}$ (ng*hr/mL)	NA	NA	396	1,870	3,190
GD 17					
C_{max} (ng/mL)	NA	NA	147	608	1,280
$AUC_{0-\tau}$ (ng*hr/mL)	NA	NA	388	3,150	6,450

Report Title: A Fertility and Embryo-fetal Development Study of RM-493 by Subcutaneous Injection in Female Rats				**Test Article:** Setmelanotide (RM-493)	
Daily Dose (mg/kg)	**0 (Vehicle)**	**0 (Saline)**	**0.5**	**3.0**	**5.0**
Prior to Mating:					
No. Evaluated	22	22	22	22	22
No. Died or Sacrificed Moribund	0	0	0	0	0
Clinical Observations	-	-	-	-	-
Premating Body Weight (%)[a]					
SD 5	258 g	-3	-6 b	-5 a	-9 ce
SD 8	262 g	-3	-8 cd	-4	-10 ce
SD 11	268 g	0	-6 ad	-3	-9 cf
SD 14	276 g	-1	-7 a	-5	-10 cf
Premating Body Weight Gain (g)					
SD 1 to 5	19 g e	12	4 ce	-1 cf	-4 cf
SD 1 to 14	37 g	36	20 ce	19 cf	11 cf
Premating Food Consumption (%)[a]					
SD 1 to 5	23.5 g	4.2	-13.2 bf	-9.6 ae	-19.3 cf
SD 1 to 14	298.8 g	3	-9 DN	-4	-10 DN
Mean No. Estrous cycles/14 days	3.5	3.2	2.9	3.1	3.2
Mean No. Days Prior to Mating	2.6	2.6	3.3	3.5	3.5
Post- Mating:					
No. of Females Mating[b]	22	22	22	22	22
No. of Pregnant Females[b]	22	19	22	21	22
No. Died or Sacrificed Moribund	0	1	0	0	0
Clinical Observations	-	-	-	-	-
Necropsy Observations (No. Examined)					
Dark focus, injection site	6 (22)	1 (20)	1 (20)	5 (21)	10 (21)

Report Title: A Fertility and Embryo-fetal Development Study of RM-493 by Subcutaneous Injection in Female Rats				**Test Article:** Setmelanotide (RM-493)	
Daily Dose (mg/kg)	**0 (Vehicle)**	**0 (Saline)**	**0.5**	**3.0**	**5.0**
Gestation Body Weight (%)[a]					
GD 0	279.3 g	-1	-6 D	-3	-9 EN
GD 3	298.5 g	0	-7 DM	-4	-10 FN
GD 6	309.6 g	0	-7 DM	-4	-11 FQ
GD 9	325.3 g	-1	-8 EM	-5	-12 FQ
GD 12	345.9 g	-1	-9 EM	-5	-12 FQ
GD 15	364.4 g	1	-9 EQ	-6	-14 FQ
GD 18	408.1 g	1	-9 EN	-7	-14 FQ
GD 21	466.4 g	1	-8 hm	-2	-9 in
Corrected GD 21	347 g	2	-6	-2	-8 be
Gestation Body Weight Gain (g)					
GD 0 to 3	19.2	21.2	15.9	15.6	12.6 DN
GD 6 to 9	15.6	12.8	9.9 D	12.8	10.3 D
GD 12 to 15	18.5	21.4	14.9 M	13.0 N	11.2 DQ
GD 15 to 18	43.8	41.1	39.3	37.5	35.2 E
GD 18 to 21	58.3	59.4	60.4	78.0 FQ	73.6 FQ
GD 6 to 18	98.5	99.3	81.9 Q	82.1 Q	73.3 FQ
Gestation Food Consumption (%)[a]					
GD 3 to 6	28.5 g	3	-9	-9	-13 DN
GD 6 to 9	28.6 g	-1	-14 EM	-7	-12 D
GD 9 to 12	29.2 g	-2	-14 EM	-7	-13 E
GD 12 to 15	29.9 g	4	-14 FQ	-10 DN	-12 EQ
GD 15 to 18	33.1 g	0	-10 DM	-7	-12 EN
GD 18 to 21	37.3 g	-11	-11	3 M	-3
GD 6 to 18	363.0 g	1	-13 FQ	-8	-13 FQ
GD 18 to 21	112.0 g	-11	-11	3 M	-3
GD 6 to 21	475.0 g	-2	-12 FN	-5 D	-10 EM

Report Title: A Fertility and Embryo-fetal Development Study of RM-493 by Subcutaneous Injection in Female Rats				**Test Article:** Setmelanotide (RM-493)	
Daily Dose (mg/kg)	**0 (Vehicle)**	**0 (Saline)**	**0.5**	**3.0**	**5.0**
Gravid Uterus Weight (%)[a]	119	-2	-14 a	-3	-11
No. with Total Resorption of Litter	0	0	0	0	0
Mean No. Corpora Lutea	17.3	16.8	15.8	16.5	15.0 y
Mean No. Implantations	16.0	16.2	13.9 x	15.1	13.9 yz
Mean % Preimplantation Loss	7.13	3.32	11.11	8.21	6.78
Litters:					
No. Litters Evaluated	22	18	20	20	21
Mean No. Live Fetuses	15.0	14.7	13.1	14.5	13.1 xz
Mean No. Resorptions	1.0	1.5	0.8	0.6	0.8
No. Dead Fetuses	0.0	0.0	0.0	0.0	0.0
Mean % Postimplantation Loss	6.15	10.44	5.08	4.14	5.47
Fetal Sex Ratios (% Male)	48.60	56.18	46.06	45.14	44.88
Mean Fetal Body Weight (g)	5.87	5.90	5.78	5.81	5.98
Fetal Anomalies:					
Gross External	-	-	-	-	-
Visceral Anomalies	-	-	-	-	-
Skeletal Anomalies	-	-	-	-	-

-, no noteworthy findings; $AUC_{0-\tau}$, area under the plasma concentration-time curve during a time interval; C_{max}, maximum plasma concentration; GD, Gestation Day; GLP, Good Laboratory Practice; NA, not applicable; SD, Study Day.

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

b Includes females where mating was only detected at necropsy.

Significantly different from mPEG-DSPE control group: a = $p \leq 0.05$, b = $p \leq 0.01$, c = $p \leq 0.001$ (t-test).
Significantly different from saline control group: d = $p \leq 0.05$, e = $p \leq 0.01$, f = $p \leq 0.001$ (t-test).
Significantly different from mPEG-DSPE control group: D = $p \leq 0.05$, E = $p \leq 0.01$, F = $p \leq 0.001$ (adjusted t-test*); g = $p \leq 0.05$, h = $p \leq 0.01$, i = $p \leq 0.001$ (adjusted Wilcoxon*).
Significantly different from saline control group: M = $p \leq 0.05$, N = $p \leq 0.01$, Q = $p \leq 0.001$, (adjusted t-test*); m = $p \leq 0.05$, n = $p \leq 0.01$, q = $p \leq 0.001$ (adjusted Wilcoxon*).
Significantly different from mPEG-DSPE control group: x = $p \leq 0.01$, y = $p \leq 0.001$ (Wilcoxon).
Significantly different from saline control group: z = $p \leq 0.05$ (Wilcoxon).
*Where p = adjusted p-value (multiplicity based on the square root of the number of pairwise comparisons).

2.6.7.13.2 ウサギを用いた胚・胎児発生に関する試験

Report Title: An Embryo-fetal Development Study of RM-493 by Subcutaneous Injection in Rabbits		**Test Article:** Setmelanotide (RM-493)
Design similar to ICH S5(R2)? Yes	**Duration of Dosing:** Group 1, GD 7 to 19 Groups 2 and 5, GD 7 to 13 Groups 3, 4 and 6, GD 13 to 19	**Study No.** RM-493-TOX-031 **Location in eCTD:** 4.2.3.5.2-2
Species/Strain: New Zealand white rabbits	**Day of Mating:** GD 0	**GLP Compliance**: Yes
	Day of C-Section: GD 29	
Initial Age: 6 months	**Method of Administration:** Subcutaneous injection	
Date of First Dose: May 2, 2016	**Vehicle/Formulation:** mPEG-DSPE	
Special Features: Dosing to cover the period of organogenesis was split between GD 7 to 13 and GD 13 to 19 for groups treated with setmelanotide; Clinical setmelanotide/mPEG-DSPE and mPEG-DSPE vehicle formulations diluted 10-fold with 5% Mannitol in sterile water for injection (USP).		
No Observed Adverse Effect Level: F_0 Females (including fertility): 0.05 mg/kg/day F_1 Litters: 0.05 mg/kg/day		

Daily Dose (mg/kg)	**0 (Control)**	**0.05**	**0.05**	**0.1**	**0.2**	**0.2**
Dosing Period	GD 7 to 19	GD 7 to 13	GD 13 to 19	GD 13 to 19	GD 7 to 13	GD 13 to 19
F_0 Females:						
Toxicokinetics						
GD 7 C_{max} (ng/mL)	NA	60.7	NA	NA	244	NA
GD 7 $AUC_{0-\tau}$ (ng*hr/mL)	NA	149	NA	NA	958	NA
GD 13 C_{max} (ng/mL)	NA	77.9	88.8	152	212	319
GD 13 $AUC_{0-\tau}$ (ng*hr/mL)	NA	214	223	524	941	1,160
GD 19 C_{max} (ng/mL)	NA	NA	53.0	139	NA	242
GD 19 $AUC_{0-\tau}$ (ng*hr/mL)	NA	NA	206	436	NA	862
Anti-Drug Antibody	NA	-	-	-	-	-

Report Title: An Embryo-fetal Development Study of RM-493 by Subcutaneous Injection in Rabbits				**Test Article:** Setmelanotide (RM-493)		
Daily Dose (mg/kg)	**0 (Control)**	**0.05**	**0.05**	**0.1**	**0.2**	**0.2**
No. Pregnant	19	20	19	22	19	21
No. Died or Sacrificed Moribund	0	0	0	0	0	0
No. Aborted or with Total Resorption of Litter	0	0	0	1	1	1
Clinical Observations						
Feces output decreased	3	4	20	21	19	21
Feces size reduced	0	0	1	5	3	4
Lesion, injection site	0	1	0	1	1	0
Necropsy Observations	-	-	-	-	-	-
Body Weight (%)[a]						
GD 13	3.4 kg	-5.6 B	0.3	-1.1	-8.5 C	-0.4
GD 20	3.6 kg	-1.8	-6.1 A	-7.6 C	-3.0	-7.8 C
GD 23	3.6 kg	-1.8	-3.5	-4.5	-3.0	-6.0 B
Body Weight Gain (kg)						
GD 7 to 13	0.1	-0.1 F	0.1	0.1	-0.2 F	0.2
GD 13 to 20	0.1	0.2	-0.1 E	-0.1 E	0.3 D	-0.2 F
GD 20 to 29	0.2	0.2	0.3 E	0.4 F	0.3	0.3
Food Consumption (%)[a]						
GD 7 to 13	997.7 g	-48 F	0	-2	-75 F	1
GD 13 to 19	1,126.3 g	-9	-72 C	-74 C	-7	-76 C
GD 20 to 29	1,164.3 g	11	15	18 A	24 B	11
Mean No. Corpora Lutea	10.4	9.9	10.5	9.6	10.2	10.4
Mean No. Implantations	9.7	9.2	9.6	9.0	9.2	9.8
Mean % Preimplantation Loss	6.78	6.80	8.03	5.97	10.42	6.80
Gravid Uterus Weight (%)[a]	531g	-6	-9	-7	-5	-14

Report Title: An Embryo-fetal Development Study of RM-493 by Subcutaneous Injection in Rabbits				**Test Article:** Setmelanotide (RM-493)		
Daily Dose (mg/kg)	**0 (Control)**	**0.05**	**0.05**	**0.1**	**0.2**	**0.2**
Litters (No. Evaluated)	19	20	19	21	18	20
No. Live Fetuses	9.5	8.9	8.8	8.3	8.7	8.5
Mean No. Resorptions	0.2	0.3	0.8	1.1	0.5	1.3
No. of Litters with Dead Fetuses	0	0	0	0	0	0
Mean % Postimplantation Loss	1.89	3.06	9.37	6.88	4.57	11.66
Mean Fetal Body Weight (g)	40.35	40.59	39.59	41.28	41.97	37.67
Fetal Sex Ratios (% live males)	52.57	49.97	50.52	55.33	54.35	49.95
Fetal Anomalies:						
Gross External	-	-	-	-	-	-
Visceral Anomalies	-	-	-	-	-	-
Skeletal Anomalies	-	-	-	-	-	-

-, no noteworthy findings; $AUC_{0-\tau}$, area under the plasma concentration-time curve during a time interval; C_{max}, maximum plasma concentration; GD, gestation day; GLP, Good Laboratory Practice; NA, not applicable

a For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences). Significantly different from vehicle control: A = p ≤0.05, B = p ≤0.01, C = p ≤0.001, (Dunnett); D = p ≤0.05; E = p ≤0.01; F = p ≤0.001 (Dunn)

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

Report Title: A Subcutaneous Injection Pre and Postnatal Development Toxicity Study of RM-493 in the Sprague-Dawley Rat				**Test Article:** Setmelanotide (RM-493)	
Design similar to ICH 4.1.2? Yes	**Duration of Dosing:** GD 6 to LD 21			**Study No.** RM-493-TOX-033 **Location in eCTD:** 4.2.3.5.3-1	
	Day of Mating: GD 0				
Species/Strain: Sprague Dawley rat	**Method of Administration:** Subcutaneous injection				
Initial Age: 11 to 13 weeks	**Vehicle/Formulation:** mPEG-DSPE			**GLP Compliance:** Yes	
Date of First Dose: October 24, 2016	**Litters Culled/Not Culled:** Litters culled to 4 pups/sex on Day 4 pp.				
Special Features: Clinical setmelanotide/mPEG-DSPE and mPEG-DSPE vehicle formulations diluted 10-fold with 5% Mannitol in sterile water for injection (USP).					
No Observed Adverse Effect Level: 5 mg/kg/day for F_0 Females and F_1 males and females					
Daily Dose (mg/kg)	**0 (mPEG-DSPE)**	**0 (Saline)**	**0.5**	**3.0**	**5.0**
F_0 Females:					
Toxicokinetics:					
Number of animals:	4	4	8	8	8
No. Died or Sacrificed Moribund	0	0	1	0	0
GD20					
Plasma AUC_{last} (ng*hr/mL)	NA	NA	231	2,050	4,140
Plasma C_{max} (ng/mL)	NA	NA	101	541	744
LD 10					
Plasma AUC_{last} (ng*hr/mL)	NA	NA	247	2,340	4,340
Plasma C_{max} (ng/mL)	NA	NA	71.2	427	990
LD 11					
Plasma Concentration (ng/mL)	NA	NA	30.7	393	636
Milk Concentration (ng/mL)	NA	NA	12.0	83.0	106

Report Title: A Subcutaneous Injection Pre and Postnatal Development Toxicity Study of RM-493 in the Sprague-Dawley Rat				**Test Article:** Setmelanotide (RM-493)	
Daily Dose (mg/kg)	**0 (mPEG-DSPE)**	**0 (Saline)**	**0.5**	**3.0**	**5.0**
No. Pregnant	**22**	**22**	**22**	**22**	**22**
No. Died or Sacrificed Moribund	0	0	0	0	1
No. Animals examined	22	22	22	22	21
No. with Total Resorption of Litter	0	0	0	0	0
Clinical Observations	-	-	-	-	-
Necropsy Observations					
Injection Site	22	22	22	22	21
Scab, dark	20	0	0	4	8
Scab, pale	6	0	0	2	2
Thin hair coat	17	0	0	8	10
Focus, dark	13	1	0	4	2
Swelling	11	0	0	1	0
Thick	12	0	0	4	3
Skin (No. Examined)	22	22	22	22	21
Scab, dark	7	0	0	1	0
Thin hair coat	3	0	0	2	4
Gestation Body Weight (%)[a]					
GD 21	436.1 g	435.4 g	-4/-4	-8A/-8 D	-13C/-13 F
Lactation Body Weight (%)[a]					
LD 21	367.8 g	360.9 g	-6/-4	-7A/-5	-12C/-10 F
Food Consumption					
GD 6-21 (%)[a]	406.1 g	420.9 g	-4/-7	-7/-10	-13A/-16 F
LD 0-14 (%)[a]	737.5 g	766.6 g	-3/-7	-7/-11 E	-19 C/-22 F
Mean Duration of Gestation (days)	21.6	21.7	21.8	21.6	21.8
Abnormal Parturition	-	-	-	-	-

Report Title: A Subcutaneous Injection Pre and Postnatal Development Toxicity Study of RM-493 in the Sprague-Dawley Rat				**Test Article:** Setmelanotide (RM-493)	
Daily Dose (mg/kg)	**0 (mPEG-DSPE)**	**0 (Saline)**	**0.5**	**3.0**	**5.0**
F_1 Litters (preweaning):					
No. Litters Evaluated	22	22	22	22	22
Mean No. Pups/Litter at Birth	13.8	12.8	13.1	13.7	13.1
Mean No. Liveborn Pups/Litter at Birth	13.8	12.7	12.9	13.7	13.1
No. of Litters with Stillborn Pups	3	2	4	1	2
Postnatal Survival to Day 4 (%)	98.56	96.63	99.43	100	94.15
Postnatal Survival to Weaning Day 21(%)	98.86	99.43	100	100	97.62
Pup Body Weights (g)					
Day 4 pp (male, female combined)	11.15 D	12.10	11.23	10.42 F	9.66 C,F
Day 21 pp (male, female combined)	57.31 D	61.41	57.84	54.78 F	49.82 C,F
Pup Sex Ratios	45.53	48.38	49.46	47.99	48.14
Pup Clinical Signs					
Thin (male/female)	0/0	1/0	0/2	0/1	6/11
Dehydrated (male/female)	0/0	0/0	0/0	0/0	6/8
Pup Necropsy Observations	-	-	-	-	-
F_1 Males (Postweaning):					
No. Died or Sacrificed Moribund	1	0	0	0	0
Clinical Observations	-	-	-	-	-
Necropsy Observations	-	-	-	-	-
Body Weight Gain Day 21 to 77 pp (g)	463.0	457.7	453.2	442.5	429.2
Food Consumption	-	-	-	-	-
Visual Function	-	-	-	-	-
Mean Age of Preputial Separation (days)	42.0	41.4	41.8	41.6	42.0
Auditory Startle Habituation	-	-	-	-	-
Motor Activity	-	-	-	-	-
Learning and Memory (Water Maze)	-	-	-	-	-
No. of Males Placed for Mating	22	22	22	22	20
Mean No. Days Prior to Mating	3.5	3.1	3.1	3.1	2.8
No. of Males that Mated	21	20	22	21	19
No. of Fertile Males	19	18	21	19	16

Report Title: A Subcutaneous Injection Pre and Postnatal Development Toxicity Study of RM-493 in the Sprague-Dawley Rat				**Test Article:** Setmelanotide (RM-493)	
Daily Dose (mg/kg)	**0 (mPEG-DSPE)**	**0 (Saline)**	**0.5**	**3.0**	**5.0**
F_1 Females (Postweaning):					
No. Evaluated	22	22	22	22	22
No. Died or Sacrificed Moribund	0	1	0	0	1/2
Clinical Observations	-	-	-	-	-
Necropsy Observations	-	-	-	-	-
Premating Body Weight Gain (g)[b]	254.5	251.0	239.7	241.0	240.3
Body Weight Gain, GD 0 to 13 (g)	67.9	72.2	71.8	69.4	65.4
Food Consumption	-	-	-	-	-
Visual Function	-	-	-	-	-
Mean Age of Vaginal Patency (days)	32.4	31.8	31.3	32.5	32.5
Auditory Startle Habituation	-	-	-	-	-
Motor Activity	-	-	-	-	-
Learning and Memory (water maze)	-	-	-	-	-
No. of Pregnant Females	19	18	21	19	16
Mean No. Corpora Lutea	18.7	18.7	19.2	18.2	18.0
Mean No. Implantations	17.7	16.7	17.3	16.9	16.3
Mean % Preimplantation Loss	5.38	12.53	10.62	7.83	8.88
F_2 Litters:					
Mean No. Live Conceptuses/Litter	16.3	15.6	15.8	15.8	15.3
Mean No. Resorptions	1.5	1.1	1.5	1.1	1.1
No. Dead Conceptuses	0	0	0	0	0
Mean % Postimplantation Loss	9.37	11.15	8.34	6.03	6.62

-, no noteworthy findings; AUC_{last}, area under the plasma concentration-time curve from time 0 to the last measurable timepoint; C_{max}, maximum plasma concentration; GD, gestation day; GLP, Good Laboratory Practice; LD, lactation day; pp, postpartum; NA, not applicable.

a For controls, group means are shown. For treated groups, percent differences from control (Group 1/Group 2) are shown. Statistical significance is based on actual data (not on the percent differences).

b From Day 21 to 77 pp.

Significantly different from mPEG=DSPE control group: A = p ≤0.05, B = p ≤0.01, C = p ≤0.001 (adjusted t-test*); a=p ≤0.05; b = p ≤0.01; c = p ≤0.001 (adjusted Wilcoxon*)

Significantly different from saline control: D = p ≤0.05 E = p ≤0.01, F = p ≤0.001 (adjusted t-test*); d = p ≤0.05; e = p ≤0.01; f = p ≤0.001 (adjusted Wilcoxon*)

*Where p = adjusted p-value (multiplicity based on the square root of the number of pairwise comparisons)

2.6.7.15 幼若動物を用いた試験

2.6.7.15.1 幼若ラットを用いた用量設定試験

Test Article: Setmelanotide (RM-493)

Species/Strain	Method of Administration (Vehicle)	Duration	Doses (mg/kg/day)[a]	Gender and No. Per Group	Noteworthy Findings	Study Number / Location in eCTD
Sprague Dawley rat	SC injection (mPD, saline)	Phase I (Subset A): 10 days (Days 7 to 16 pp) Phase I (Subset B): 10 days (Days 21 to 30 pp) Phase II: 4 weeks (Days 7 to 34 pp)	Phase I: 0 (mPD), 2, 5, 15 (in saline), 2, 4 (in mPD) Phase II: 0 (mPD), 15 (in saline), 1, 2.5, 5 (in mPD)	Phase I: 2/sex Phase II: Main: 8/sex TK: 2 or 6/sex	For the tolerability phase, 15 mg/kg/day (in saline) and doses up to 4 mg/kg/day (in mPD) were well tolerated. Skin redness and/or scabbing at the injection site were observed for the first few days of dosing only in all pups at 15 mg/kg/day (in saline), and there were lower, non dose-dependent, body weight gains between Days 7 and 10 pp (Subset A) or Days 21 up to 31 pp (Subset B) in males and females in all RM-493-treated groups. For the 28-day dose range finding phase, body weights at 5 mg/kg/day (in mPD) were lower compared to controls at the end of the dosing period, and a Test Item-related decrease in food consumption at 5 mg/kg/day was noted compared to controls from Days 24 to 34 pp. Slight decreases in food consumption were also noted from Days 24 to 34 pp in males and females at 2.5 mg/kg/day (in mPD) and 15 mg/kg/day (in saline). There were no RM-493-related clinical signs. 15 mg/kg/day (in saline) produced modest decreases in body weight and was well tolerated at the injection sites, as no RM-493-related gross findings were noted. 5 mg/kg/day was considered to have exceeded the maximum tolerated dose.	RM-493-TOX-034 / 4.2.3.5.4-1

mPD, mPEG-DSPE; pp, post partum; SC, subcutaneous; TK, toxicokinetic

2.6.7.15.2 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

Report Title: A 7 -Week Subcutaneous Injection Toxicity Study of RM-493 in the Juvenile Rat with a 4 Week Recovery Period		**Test Article**: Setmelanotide (RM-493)
Design similar to ICH S3a?: Yes	**Duration of Dosing**: Day 7 to 55 pp	**Study No.** RM-493-TOX-035
Species/Strain: Rat / Crl:CD(SD) Sprague-Dawley	**Day of Mating**: Day 0 post coitum	**Location in CTD**: 4.2.3.5.4-2
Initial Age: 7 day old at start of dosing	**Day of C-Section**: GD 13 **Duration of Postdose**: 4 Weeks	**GLP Compliance**: Yes
Date of First Dose: 25 Apr 2016	**Method of Administration**: Subcutaneous injection	
Special Features: Toxicokinetics, Ophthalmology, Clinical pathology, Bone measurements	Formulation[a]: Group 1 (Vehicle Control): RM-493 mPEG/DSPE Placebo (mPEG/DSPE diluted 1:10 in 5% Mannitol in sterile water for injection (USP)) Group 2 (Saline Control): 0.9% Sodium Chloride Injection, USP	
No Observed Adverse Effect Level:	RM-493 in mPEG/DSPE: 3 mg/kg/day RM-493 in Saline: 15 mg/kg/day	

Dose (mg/kg)[a]	**0 (Vehicle)**		**0 (Saline)**		**0.5**		**1.5**		**3**		**15**	
Number of Animals/Sex/Group:	M	F	M	F	M	F	M	F	M	F	M	F
Toxicokinetic[b]:	8	8	4	4	36	36	36	36	36	36	36	36
Toxicokinetics												
Day 7 pp												
C_{max} (ng/mL)	-		-		179		510		861		4540	
AUC_{0-t} (ng*hr/mL)	-		-		429		1580		2920		9210	
Day 55 pp												
C_{max} (ng/mL)	-		-		158		421		805		3560	
AUC_{0-t} (ng*hr/mL)	-		-		452		1760		3840		18800	

pp, post partum; GD, Gestation Day; M, Male; F, Female.

a Formulations for Groups 1, 3, 4, and 5 were diluted in 5% Mannitol. Formulation for Group 6 was diluted in saline.

b Toxicokinetic parameters presented for males and females combined.

Report Title: A 7 -Week Subcutaneous Injection Toxicity Study of RM-493 in the Juvenile Rat with a 4 Week Recovery Period										**Test Article**: Setmelanotide (RM-493)		
Dose (mg/kg)[a]	**0 (Vehicle)**		**0 (Saline)**		**0.5**		**1.5**		**3**		**15**	
Number of Animals/Sex/Group:	M	F	M	F	M	F	M	F	M	F	M	F
Main:	12	12	12	12	12	12	12	12	12	12	12	12
Recovery:	12	12	12	12	12	12	12	12	12	12	12	12
Neuro/Repro:	20	20	20	20	20	20	20	20	20	20	20	20
Noteworthy Findings												
Pre-Weaning												
Died or Euthanized Moribund	0	0	0	0	1	1	0	0	0	0	0	0
Pup Clinical Observations	-	-	-	-	-	-	-	-	-	-	-	-
Body Weight (%[b])												
Day 21 pp	55.1	53.4	0	0	1	0	-6[DM]	-5	-5	-6[DM]	-7[M]	-5
Body Weight Gains (g)												
Day 7 to 10 pp	8.3	8.3	8.6	8.4	8.0[m]	7.8[m]	8.0[m]	7.6[m]	7.9[n]	7.4[n]	7.5[q]	7.4[n]
Day 17 to 21 pp	10.6	10.2	9.3	9.7	11.0[M]	10.5	8.6[E]	9.1	7.9[F]	7.7[FN]	8.4	8.8
Day 7 to 21 pp	36.9	35.7	36.7	36.2	36.8	35.7	34.0[EM]	33.6[M]	34.3[DM]	32.9[EN]	33.2[Q]	33.3[N]
Post-Weaning												
Died or Euthanized Moribund	0	0	0	0	0	0	0	0	0	1	0	0
Clinical Observations	-	-	-	-	-	-	-	-	-	-	-	-
Body Weight (g) (%[b])												
Day 24 pp	70.5	68.7	0	-2	2	-1	-7[DM]	-8[EM]	-6[DM]	-11[FQ]	-10[Q]	-10[N]
Day 28 pp	99.5	94.2	1	-1	3	0	-4	-4	-4	-7[EN]	-8[Q]	-8[N]
Day 31 pp	125.6	116.4	1	-1	3	1	-3	-3	-2	-6[E]	-6[N]	-6
Day 35 pp	165.9	148.4	2	-2	5	1	-2	-1	-1	-4	-5[N]	-5
Day 38 pp	199.3	170.8	2	-2	4	1	-2	0	-1	-4	-4[M]	-4
Day 42 pp	243.7	196.3	2	-2	4	1	-2	1	0	-2	-3[M]	-2
Day 45 pp	276.0	211.4	2	-3	4	1	-2	1	0	-2	-2	-1
Body Weight Gains (g)												
Day 21 to 24 pp	15.3	15.4	15.2	13.6	16.7	14.7	14.2	12.3[F]	13.7	11.3[FN]	12.0[Q]	11.3[N]
Day 21 to 52 pp	290.5	188.9	298.8	180.7	306.8	194.4[N]	292.3	193.1[N]	298.0	187.6	290.5	188.0
Food Consumption (g) (%[b])												
Day 21 to 24 pp	7.9	7.9	-4	1	4	-2	1	2	-4	-15	-13	-13
Day 21 to 52 pp	708.3	552.7	1	-2	2	4	1	4	-2	2	-4	-2

Report Title: A 7 -Week Subcutaneous Injection Toxicity Study of RM-493 in the Juvenile Rat with a 4 Week Recovery Period										**Test Article**: Setmelanotide (RM-493)		
Dose (mg/kg)[a]	**0 (Vehicle)**		**0 (Saline)**		**0.5**		**1.5**		**3**		**15**	
Number of Animals/Sex/Group:	M	F	M	F	M	F	M	F	M	F	M	F
Main:	12	12	12	12	12	12	12	12	12	12	12	12
Recovery:	12	12	12	12	12	12	12	12	12	12	12	12
Neuro/Repro:	20	20	20	20	20	20	20	20	20	20	20	20
Gross Pathology												
Site, Injection; Focus; dark	3	3	5	3	4	3	7	3	9	5	11	9
Histopathology												
Injection Site;												
Hemorrhage												
Minimal	4	3	2	1	2	2	8	1	5	3	5	7
Mild	1	0	0	1	1	0	2	1	2	2	6	2
Moderate	0	0	1	0	0	0	0	0	1	0	0	0
Severe	0	0	0	0	0	0	0	0	0	0	0	1
Infiltration, mixed cell												
Minimal	2	6	3	3	5	7	6	6	3	6	5	6
Mild	10	5	1	0	2	0	6	0	8	5	6	6
Moderate	0	1	0	0	0	0	0	0	1	0	0	0
Degeneration / Regeneration; myofiber												
Minimal	4	2	0	0	0	2	1	1	1	1	4	4
Mild	1	2	0	0	1	0	0	0	0	1	3	6
Moderate	0	1	0	0	0	0	1	0	0	0	0	0
Crust												
Minimal	0	0	0	0	1	0	2	0	0	0	4	0
Recovery												
Died or Euthanized Moribund	0	0	0	0	0	0	0	0	0	0	0	0
Clinical Observations	-	-	-	-	-	-	-	-	-	-	-	-

Report Title: A 7 -Week Subcutaneous Injection Toxicity Study of RM-493 in the Juvenile Rat with a 4 Week Recovery Period											**Test Article**: Setmelanotide (RM-493)	
Dose (mg/kg)[a]	**0 (Vehicle)**		**0 (Saline)**		**0.5**		**1.5**		**3**		**15**	
Number of Animals/Sex/Group:	M	F	M	F	M	F	M	F	M	F	M	F
Main:	12	12	12	12	12	12	12	12	12	12	12	12
Recovery:	12	12	12	12	12	12	12	12	12	12	12	12
Neuro/Repro:	20	20	20	20	20	20	20	20	20	20	20	20
Body Weight (g) (%[b])												
Day 59 pp	414.2	274.0	3	-2	5	2	3	5^{N}	6	1	5	5^{N}
Day 63 pp	451.0	285.5	2	-1	5	3	4	6^{DN}	7^{E}	3	7	7^{Q}
Day 66 pp	478.0	295.7	2	-2	5	3	4	6^{DN}	8^{EM}	3	7	8^{Q}
Day 70 pp	510.4	305.1	1	-2	5	3	5	6^{DN}	9^{EN}	3	8^{M}	8^{Q}
Day 73 pp	530.8	311.8	1	-2	6	4^{M}	5	6^{DN}	9^{FN}	3^{M}	9^{N}	9^{Q}
Day 77 pp	563.6	323.6	1	-2	6	3	5	6^{DN}	9^{FN}	4^{M}	9^{N}	9^{Q}
Day 80 pp	570.0	323.8	1	-1	6^{D}	4	6^{D}	8^{EQ}	10^{FQ}	5^{M}	10^{Q}	10^{Q}
Body Weight Gains (g)												
Day 56 to 80	182.1	62.4	180.3	68.5	208.6^{FQ}	76.2^{f}	216.0^{FQ}	84.4^{fq}	234.9^{FQ}	85.7^{fq}	237.1^{Q}	97.8^{q}
Food Consumption (g) (%[b])												
Day 56 to 83	1039.7	649.1	-2	-6	4	4	16	8	9	6	13	17
Histopathology												
Site, Injection												
Infiltration, mixed cell												
Minimal	1	0	0	0	0	0	1	0	3	0	6	1
Main/Recovery												
Sexual Maturation	-	-	-	-	-	-	-	-	-	-	-	-
Growth (Crown to Rump)	-	-	-	-	-	-	-	-	-	-	-	-
Behavioral Performance	-	-	-	-	-	-	-	-	-	-	-	-
Behaviour and Memory	-	-	-	-	-	-	-	-	-	-	-	-
Ophthalmic Examinations	-	-	-	-	-	-	-	-	-	-	-	-
Organ Weights	-	-	-	-	-	-	-	-	-	-	-	-
Coagulation(%[b])												
FIB (mg/dL)												
Day 56 pp	335.5^{v}	261.3	-17	-10	11	-9	27^{dq}	-6	29^{eq}	0	18^{eq}	-5
Day 84 pp	276.5	219.7	2	-4	1	1	11^{D}	2	14^{DM}	2	11	9

Report Title: A 7 -Week Subcutaneous Injection Toxicity Study of RM-493 in the Juvenile Rat with a 4 Week Recovery Period											**Test Article**: Setmelanotide (RM-493)	
Dose (mg/kg)[a]	**0 (Vehicle)**		**0 (Saline)**		**0.5**		**1.5**		**3**		**15**	
Number of Animals/Sex/Group:	M	F	M	F	M	F	M	F	M	F	M	F
Main:	12	12	12	12	12	12	12	12	12	12	12	12
Recovery:	12	12	12	12	12	12	12	12	12	12	12	12
Neuro/Repro:	20	20	20	20	20	20	20	20	20	20	20	20
Clinical Chemistry(%[b])												
TRIG (mg/dL)												
Day 56 pp	60.3	32.6	-23	-7	-7	0	-7	-5	-18	-9	-23	-6
Day 84 pp	53.5	31.8	-6	-5	20	39[M]	126[en]	33	65[m]	57[EN]	70[n]	64[Q]
ALB (g/dL)												
Day 56 pp	3.59[B]	4.10	6	1	-2[Q]	2	-6[DQ]	-1	-5[DQ]	2	0[N]	1
Day 84 pp	3.92	4.43	-1	-3	0	-4	-1	1	-2	1	-1	-2
GLOB (g/dL)												
Day 56 pp	1.80	1.67	-5	1	3	5	5[M]	1	6[M]	1	0	3
Day 84 pp	2.05	1.95	1	-2	5	1	9	8[M]	9	4	8	7[M]
A/G												
Day 56 pp	2.01[A]	2.50	12	-1	-5[Q]	-3	-10[DQ]	-3	-10[Q]	0	-1[N]	-2
Day 84 pp	1.93	2.29	-2	-1	-5	-5	-10	-7	-10	-3	-7	-8
Neuro/Repro Performance												
Body Weight (g)(%[b])												
Day 87 pp	577.9	N/A	4	N/A	9[D]	N/A	4	N/A	13[FM]	N/A	12[M]	N/A
Day 91 pp	600.5	N/A	4	N/A	8[D]	N/A	5	N/A	13[FM]	N/A	12[M]	N/A
Day 94 pp	612.8	N/A	3	N/A	9[D]	N/A	5	N/A	13[FN]	N/A	13[M]	N/A
Day 98 pp	633.4	N/A	3	N/A	9[D]	N/A	5	N/A	13[FN]	N/A	12[N]	N/A
Day 101 pp	644.3	N/A	3	N/A	9[D]	N/A	5	N/A	14[FN]	N/A	13[N]	N/A
Day 105 pp	654.9	N/A	3	N/A	10[E]	N/A	6	N/A	15[FN]	N/A	13[N]	N/A
Day 108 pp	668.0	N/A	3	N/A	10[D]	N/A	6	N/A	15[FQ]	N/A	13[N]	N/A
Day 112 pp	679.2	N/A	3	N/A	10[E]	N/A	7	N/A	16[FQ]	N/A	14[N]	N/A
Gestating Females												
Body Weight (g)(%[b])												
GD 0	N/A	329.8	N/A	1	N/A	6	N/A	7	N/A	8	N/A	9[M]
GD 7	N/A	377.9	N/A	0	N/A	6	N/A	6	N/A	9[DM]	N/A	8[M]
GD 13	N/A	420.8	N/A	-1	N/A	6	N/A	5	N/A	8[DM]	N/A	7

Report Title: A 7 -Week Subcutaneous Injection Toxicity Study of RM-493 in the Juvenile Rat with a 4 Week Recovery Period											**Test Article**: Setmelanotide (RM-493)	
Dose (mg/kg)[a]	**0 (Vehicle)**		**0 (Saline)**		**0.5**		**1.5**		**3**		**15**	
Number of Animals/Sex/Group:	M	F	M	F	M	F	M	F	M	F	M	F
Main:	12	12	12	12	12	12	12	12	12	12	12	12
Recovery:	12	12	12	12	12	12	12	12	12	12	12	12
Neuro/Repro:	20	20	20	20	20	20	20	20	20	20	20	20
Body Weight Gains (g)												
GD 0 to 7	N/A	48.1	N/A	45.4	N/A	49.3	N/A	48.6	N/A	54.3	N/A	47.6
GD 7 to 13	N/A	42.9	N/A	38.6	N/A	45.4	N/A	41.8	N/A	45.0	N/A	42.2
Mean No. Days Prior to Mating	2.8		2.9		4.1		4.3		3.1		3.0	
No. of Animals that Mated	18	18	20	20	19	19	18	18	19	19	16	16
No. of Females Sperm-Positive	N/A	18	N/A	20	N/A	19	N/A	18	N/A	19	N/A	16
No. of Pregnant Females	N/A	16	N/A	18	N/A	18	N/A	17	N/A	17	N/A	14
Mean No. Corpora Lutea[c]	N/A	17.9	N/A	16.1	N/A	18.1	N/A	18.8	N/A	17.5	N/A	18.1
Mean No. Implantations[c]	N/A	16.7	N/A	14.8	N/A	16.9	N/A	16.7	N/A	16.3	N/A	16.2
Mean % Preimplantation Loss[c]	N/A	7.3	N/A	8.6	N/A	7.4	N/A	11.4	N/A	9.2	N/A	10.5
Mean % Postimplantation Loss[c]	N/A	4.2	N/A	4.7	N/A	2.1	N/A	7.7	N/A	12.1	N/A	8.9
No. with Total Resorption of Litter	N/A	0	N/A	0	N/A	0	N/A	0	N/A	1	N/A	0
Number of Live Embryos[c]	N/A	16.1	N/A	14.1	N/A	16.6	N/A	15.4	N/A	15.2	N/A	14.7
Mean No. Resorptions & Dead Embryos[c]	N/A	0.5	N/A	0.7	N/A	0.4	N/A	1.3	N/A	1.1	N/A	1.5
Gross Pathology	-	-	-	-	-	-	-	-	-	-	-	-

-, No noteworthy findings; GD, Gestation Day; pp, post partum; M, Male; F, Female.
a Formulations for Groups 1, 3, 4, and 5 were diluted in 5% Mannitol. Formulation for Group 6 was diluted in saline.
b For control Group 1, group means are shown. For control Group 2 and treated groups, percent differences from Group 1 are shown.
c Including animal with total resorption.
Statistical significance is based on actual data (not on the percent differences).
Group 3 to 5 Significantly different from control group (Group 1) value: D - P' ≤ 0.05; E - P' ≤ 0.01; F - P' ≤ 0.001 (adjusted t-test)
d - P' ≤ 0.05; e - P' ≤ 0.01; f - P' ≤ 0.001 (adjusted Wilcoxon)
Group 3 to 6 Significantly different from control group (Group 2) value: M - P' ≤ 0.05; N - P' ≤ 0.01; Q - P' ≤ 0.001 (adjusted t-test)
m - P' ≤ 0.05; n - P' ≤ 0.01; q - P' ≤ 0.001 (adjusted Wilcoxon)
Group 1 Significantly different from control group (Group 2) value: A - P' ≤ 0.05; B - P' ≤ 0.01; C - P' ≤ 0.001 (adjusted t-test)
u - P' ≤ 0.05; v - P' ≤ 0.01; w - P' ≤ 0.001 (adjusted Wilcoxon)
Where P' = adjusted p-value (multiplicity based on the square root of the number of pairwise comparisons).
Statistical significance is based on actual data (not on the percent differences).

2.6.7.16 局所刺激性試験

					Test Article: Setmelanotide (RM-493)	
Species/Strain	**Method of Administration (Vehicle/Formulation)**	**Duration**	**Doses (mg/kg/dose)[a]**	**Gender and No. Per Group**	**Noteworthy Findings**	**Study Number / Location in eCTD**
Sprague Dawley rat	SC injection (mPEG-DSPE)	7 days	0, 5, 10 (10 mg/mL)	10 males	All groups including vehicle control: Minimal to moderate inflammation of the subcutaneous space, minimal degenerative changes of the myofibers, and/or necrotic changes at injection sites.	RM-493-TOX-022 / 4.2.3.6-1

SC, subcutaneous

a Setmelanotide concentration in dose formulations are shown in parenthesis.

2.6.7.17 その他の毒性試験

Test Article: Setmelanotide (RM-493)							
Species/ Strain	**Method of Administration (Vehicle)**	**Duration of Dosing**	**Doses (mg/kg/day)[a]**	**Gender and No. Per Group**	**NOAEL (mg/kg/day)**	**Noteworthy Findings**	**Study Number / Location in eCTD**
Sprague-Dawley rat	SC infusion (saline)	13 weeks	0 (saline), 10, 30, 60	Main:15/sex Recovery: 5/sex (control and high dose) TK: 9/sex	30	Body weight reductions correlating with decreased food consumption were observed at 10, 30, 60 mg/kg/day. Microscopic findings that included reversible adrenal cortical hypertrophy in all RM-493 dose groups, epidermal ulceration at the infusion site in males at 30 and 60 mg/kg/day, and increased severity of chronic inflammation for males at the infusion site at 60 mg/kg/day were observed. Ventricular and atrial dilatation was noted grossly in 1 male given 60 mg/kg/day which correlated with ventricular and atrial dilatation and ventricular wall thickening in this animal and may have been RM-493 related. Changes considered secondary to the microscopic findings were observed in clinical signs (red/blue/white skin, discharge and skin scab/lesion at the surgical or lumbar sites with a dose related increase) and hematology changes noted at all RM-493 doses but primarily at 60 mg/kg/day that included minimal increases (statistically significant) in mean percent and/or absolute neutrophils and reticulocytes, monocytes, eosinophils and decreased lymphocytes. These changes were not observed at the end of the recovery period.	RM-493-TOX-016 / 4.2.3.7.7-1

Test Article: Setmelanotide (RM-493)							
Species/ Strain	**Method of Administration (Vehicle)**	**Duration of Dosing**	**Doses (mg/kg/day)[a]**	**Gender and No. Per Group**	**NOAEL (mg/kg/day)**	**Noteworthy Findings**	**Study Number / Location in eCTD**
Cynomolgus monkey	SC infusion (saline)	13 weeks	0 (saline), 1, 10, 25	Main: 4/sex Recovery: 2/sex (control and high dose)	25	RM-493 related findings including epidermal pigment (consistent with melanin) in the skin of the muzzle and lower lip at ≥ 1 mg/kg/day and local effects at the infusion site at ≥ 1 mg/kg/day including thickening/ firmness and/or masses and inflammation were observed. Based on immunohistochemistry against S-100 protein, which is expressed by melanocytes and not by keratinocytes, there was no noticeable difference between controls and RM-493 treated animals in the amount of positive cells in the epidermis of the skin of the muzzle. At the end of the 10-week recovery period, a decreased incidence and severity of epidermal pigment in the skin of the muzzle and lower lip and a decreased severity of the inflammation at the infusion site suggest ongoing resolution of these changes. With the exception of reactions at the infusion site and skin hyperpigmentation, all dose levels were well tolerated with no indication of systemic organ toxicity at doses up to 25 mg/kg/day.	RM-493-TOX-015 / 4.2.3.7.7-2