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（試験番号 [redacted]）

Study Title: Bacterial Reverse Mutation Assay			
GLP	Yes	Test Article: AR-13324	
Location	4.2.3.3.1-1	Date of Experiment	[redacted] 20[redacted] 20[redacted]
Test for Induction of	Reverse mutation in bacterial cells	No. of Independent Assays	Duplicate, initial assay or retest and confirmatory assay
Strains	*Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) *Escherichia coli* (strain WP2 *uvrA*)	No. of Replicate Cultures	Triplicate
Metabolizing System	Aroclor 1254-induced rat liver S9 (Sprague-Dawley)	No. of Cells Analyzed/Culture	$\geq 0.3 \times 10^9$ cells/mL
Vehicle For Test Article	Distilled Water	Vehicle For Positive Controls	DMSO, except water for sodium azide
Treatment	Plate incorporation - Incubation for 48-72 hours at 37±2°C		
Cytotoxic Effects	Initial Assay: Growth inhibition in TA98 and TA1535 above 50 µg/plate, in TA100 and TA1537 above 15 µg/plate, in WP2*uvr*A above 500 µg/plate without activation. Growth inhibition in TA98, TA1535 and WP2*uvr*A above 500 µg/plate, in TA100 and TA1537 above 150 µg/plate with activation. Retest: Growth inhibition in TA100 above 20 µg/plate, in TA1537 above 60 µg/plate without activation. Confirmatory Assay: Growth inhibition in TA98 above 20 µg/plate, in TA100, TA1535 and TA1537 above 60 µg/plate, in WP2*uvr*A above 200 µg/plate without activation. Growth inhibition in TA98, TA100, TA1535 and TA1537 above 200 µg/plate, in WP2*uvr*A 600 µg/plate with S9 activation.		
Genotoxic Effects	None		

Confidential

2.6.7.8.1 細菌を用いる復帰突然変異試験（試験番号 [redacted]）（続き）

Study Title: Bacterial Reverse Mutation Assay							
Metabolic Activation	Test Article	Dose Level (μg/plate)	Initial Assay: Revertant Colony Counts (Mean ± SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without Activation	Netarsudil	5000	0±0	0±0	0±0	0±0	0±0
		1500	0±0	0±0	0±0	0±0	0±0
		500	0±0	0±0	0±0	0±0	0±0
		150	0±0	0±0	0±0	0±0	40±2
		50	0±0	0±0	0±0	0±0	41±1
		15	20±5	0±0	14±2	0±0	44±3
		5.0	13±3	126±6	14±2	8±0	44±24
		1.5	17±6	122±6	14±1	7±3	43±2
	Water	100 μL/plate	19±2	142±17	10±1	5±1	47±7
	2-nitrofluorene	1.0	283±78				
	Sodium azide	1.0		982±65	706±4		
	9-aminoacridine	75				958±256	
	Methyl methanesulfonate	1000					441±9

Confidential

2.6.7.8.1 細菌を用いる復帰突然変異試験（試験番号 [redacted]）（続き）

Study Title: Bacterial Reverse Mutation Assay							
Metabolic Activation	Test Article	Dose Level (μg/plate)	Initial Assay: Revertant Colony Counts (Mean ± SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
With Activation	Netarsudil	5000	0±0	0±0	0±0	0±0	0±0
		1500	0±0	0±0	0±0	0±0	0±0
		500	0±0	0±0	0±0	0±0	0±0
		150	26±4	0±0	20±1	0±0	38±3
		50	29±0	158±18	16±6	15±6	48±13
		15	24±1	139±6	25±1	9±6	36±3
		5.0	25±1	160±18	18±4	20±1	35±3
		1.5	21±6	153±3	19±4	5±1	42±11
	Water	100 μL/plate	24±1	171±10	23±3	11±3	34±10
	2-aminoanthracene	1.0	347±192		75±2	52±8	
	2-aminoanthracene	2.0		690±115			
	2-aminoanthracene	15					298±14

Confidential

2.6.7.8.1 細菌を用いる復帰突然変異試験（試験番号 [redacted]）（続き）

Study Title: Bacterial Reverse Mutation Assay							
Metabolic Activation	Test Article	Dose Level (μg/plate)	Retest[1]: Revertant Colony Counts (Mean ± SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without Activation	Netarsudil	60		0±0		0±0	
		20		0±0		6±3	
		6.0		101±4		10±3	
		2.0		98±1		9±3	
		0.60		105±9		13±1	
		0.20		97±12		11±3	
	Water	100 μL/plate		99±8		10±3	
	Sodium azide	1.0		777±35			
	9-aminoacridine	75				604±232	
Metabolic Activation	Test Article	Dose Level[2] (μg/plate)	Confirmatory Assay: Revertant Colony Counts (Mean ± SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without Activation	Netarsudil	60 / 600	0±0	0±0	0±0	0±0	0±0
		20 / 200	0±0	71±11	7±2	6±2	0±0
		6.0 / 60	17±4	115±13	13±5	7±3	37±4
		2.0 / 20	10±3	102±5	10±3	3±2	41±6
		0.60 / 6.0	17±6	108±2	12±3	7±2	45±3
		0.20 / 2.0	15±2	104±6	12±3	4±2	41±5

1: Due to excessive toxicity, TA100 and TA1537 in the absence of S9 activation were not evaluated for mutagenicity but were retested based on the findings of the initial toxicity-mutation assay.

2: Based on findings of initial assay, maximum doses in confirmatory assays were 60 μg per plate with all Salmonella tester strains in the absence of S9 activation and 600 μg per plate with WP2*uvr*A.

Confidential

2.6.7.8.1 細菌を用いる復帰突然変異試験（試験番号 [redacted]）（続き）

Study Title: Bacterial Reverse Mutation Assay							
Metabolic Activation	Test Article	Dose Level (μg/plate)	Confirmatory Assay: Revertant Colony Counts (Mean ± SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without Activation	Water	100 μL/plate	13±3	104±10	10±2	7±1	48±9
	2-nitrofluorene	1.0	173±33				
	Sodium azide	1.0		631±50	535±46		
	9-aminoacridine	75				244±22	
	Methyl methanesulfonate	1000					316±95
With Activation	Netarsudil	600	0±0	0±0	0±0	0±0	0±0
		200	0±0	0±0	0±0	0±0	31±3
		60	12±2	123±3	13±4	8±3	51±12
		20	17±2	132±8	15±5	7±1	43±3
		6.0	9±4	136±6	9±2	7±2	51±9
		2.0	11±3	139±5	14±1	10±4	47±5
	Water	100 μL/plate	15±4	132±19	19±5	7±2	39±5
	2-aminoanthracene	1.0	475±95		117±4	71±7	
	2-aminoanthracene	2.0		978±501			
	2-aminoanthracene	15					242±20

Confidential

2.6.7.8.2 マウスリンフォーマ TK 試験（試験番号 [redacted]）

Study Title: *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)			
GLP	Yes	Test Article: AR-13324	
Location	4.2.3.3.1-2	Date of Experiment	[redacted] 20[redacted] 20[redacted]
Test for Induction of	Mutagenesis	No. of Independent Assays	Duplicate, preliminary and extended confirmatory
Strains	L5178Y (TK$^{+/-}$ -3.7.2c) mouse lymphoma cells	No. of Replicate Cultures	Each plated in triplicate
		No. of Cells Analyzed/Culture	3x10^5 cells/mL
Vehicle For Test Article	Distilled water	Vehicle For Positive Controls	Distilled water for methyl methanesulfonate, DMSO for 7,12-Dimethyl-benz(a)anthracene
Metabolizing System	Aroclor 1254-induced rat liver S9	Treatment	4-hour exposure with and without S9 activation, 24-hour exposure without S9 activation
Genotoxic Effects	None	Cytotoxic Effects	Suspension growth relative to the solvent controls was 0% at ≥5.0 µg/mL with and without activation with a 4 -hour exposure and without activation with a 24-hour exposure.

Metabolic Activation	Test Article	4 Hour Exposure			24 Hour Exposure		
		Concentration (µg/mL)	% Relative Total Growth	Induced mutant frequency (/10^6 cells)	Concentration (µg/mL)	% Relative Total Growth	Induced mutant frequency (/10^6 cells)
Without Activation	Netarsudil	0.1	123, 108	31, 6	0.5	81, 96	12, -8
		0.5	73, 71	-3, -11	0.7	72, NC	-1, NC
		1.0	57, 58	-18, 4	0.9	56, 61	13, 0
		1.5	21, 14	-28, -36	1.0	39, 38	-1, 6
		2.0	3	NC	1.1	18, 16	5, 13
	MMS	20	4	635	7.5	14	669
		15	17	342	5	33	390

MMS: Methyl methanesulfonate, NC: Not calculated

Confidential

2.6.7.8.2 マウスリンフォーマ TK 試験（試験番号 [redacted]）（続き）

Study Title: *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)							
Metabolic Activation	Test Article	4 Hour Exposure			24 Hour Exposure		
		Concentration (μg/mL)	% Relative Total Growth	Induced mutant frequency (/10^6 cells)			
With Activation	Netarsudil	3	69, 92	14, -20			
		3.5	57, 59	33, 8			
		4.5	60, 55	22, 32			
		5	54, 57	2, 60			
		7	28, 19	-25, -4			
	7,12-DMBA	1.5	7	415			
		1.25	14	334			

7,12-DMBA: 7,12-Dimethyl-benz(a)anthracene

Confidential

2.6.7.9 *In Vivo* 遺伝毒性試験

2.6.7.9.1 ラット小核試験（試験番号 [redacted]）

Study Title: A 7-Day GLP Intravenous Toxicity Study of AR-13324 in Rats with a 7-Day Recovery							
GLP	Yes			Test Article: AR-13324			
Location	4.2.3.3.2-1			Date of First Dose		[redacted] 20[redacted] (Males), [redacted] 20[redacted] (Females)	
Test for Induction of	Bone marrow micronuclei			Treatment Schedule		7 days, once daily	
Species/Strain	Rat / Sprague-Dawley			Sampling Time		Day 8 and 15	
Age	6-7 weeks			No. of Cells Analyzed/Animal		2000 PCEs, 1000 erythrocytes/ field	
Method of Administration	Intravenous			Cells Evaluated		Polychromatic erythrocytes (PCEs)	
Vehicle/Formulation	0.9% NaCl/Solution			Special Features		None	
Toxic Effects	3 and 10 mg/kg caused hematology findings and microscopic lesions in the liver that were consistent with anemia and considered adverse effects. Injection site reactions in the 10 mg/kg group still present at end of recovery						
Genotoxic Effects	None						
Treatment	Sex	Day	Number of Animals	PCE/Total Erythrocytes (Mean ± SD)	Change from Control (%)	Number of MPCE/1000 PCE (Mean ± SD)	Number of MPCE/PCE Scored
0 mg/kg/day	M	8	5	0.487±0.05	-	0.6±0.42	6/10000
1 mg/kg/day	M	8	5	0.462±0.06	-5	0.3±0.45	3/10000
3 mg/kg/day	M	8	5	0.451±0.03	-7	0.2±0.27	2/10000
10 mg/kg/day	M	8	5	0.435±0.09	-11	0.4±0.42	4/10000

PCE: Polychromatic erythrocytes, MPCEs: Micronucleated polychromatic erythrocytes

Confidential

2.6.7.9.1 ラット小核試験（試験番号 [redacted]）（続き）

Study Title: A 7-Day GLP Intravenous Toxicity Study of AR-13324 in Rats with a 7-Day Recovery							
Treatment	Sex	Day	Number of Animals	PCE/Total Erythrocytes (Mean ± SD)	Change from Control (%)	Number of MPCE/1000 PCE (Mean ± SD)	Number of MPCE/PCE Scored
Recovery Period[1]							
0 mg/kg/day	M	15	5	0.453±0.06	-	0.4±0.22	4/10000
10 mg/kg/day	M	15	5	0.464±0.03	2	0.3±0.45	3/10000

PCE: Polychromatic erythrocytes, MPCEs: Micronucleated polychromatic erythrocytes

1: Bone marrow smears were prepared after a recovery period of 1 week, on Study Day 15 post-dose.

Confidential

2.6.7.10 がん原性試験

該当資料なし。

Confidential

2.6.7.11 生殖発生毒性試験： 重要な試験以外の試験

Test Article: AR-13324

Species / Strain	Method of Administration (Vehicle/ Formulation)	Dosing Period	Doses (mg/kg/day)	No. per group	Noteworthy Findings	Study Number
Rat / Sprague Dawley	Intravenous (0.9% NaCl/Solution)	Gestation Day 6 to 17	Intended (Protocol): 0, 0.3, 3, 10 Measured: 0, 0.09, 2.4, 10	Vehicle: 5 AR-13324: 7	10 mg/kg/day was adverse to developing embryo and fetus (high mortality). The 3 mg/kg/day was moderately adverse to both embryo and fetal survival, but not to the extent fetal morphological variations and/or malformations could not be assessed. 3 mg/kg/day recommended as high dose for embryo-fetal development in rat study.	[redacted] (Non-GLP)
Rabbit / New Zealand White	Intravenous (0.9% NaCl/Solution)	Gestation Day 6 through 19	Intended (Protocol): 0, 0.1, 0.3, 1, 3 Measured: 0, 0.08, 0.24, 0.8, 3	7	No significant changes in most study measures. Subtle differences in several cesarean parameters at 3 mg/kg/day versus control and the other test article groups: a slightly higher mean number of nonviable fetuses, a lower mean percentage of viable fetuses, a higher mean percentage of nonviable fetuses, and a lower mean fetal weight and mean uterine weight compared to the corresponding parameters of the vehicle control and other test article-treated groups. AR-13324 at ≤ 3 mg/kg/day administered intravenously to pregnant rabbits were well tolerated.	[redacted] (Non-GLP)

Confidential

2.6.7.11 生殖発生毒性試験： 重要な試験以外の試験（続き）

Test Article: AR-13324						
Species / Strain	Method of Administration (Vehicle/ Formulation)	Dosing Period	Doses (mg/kg/day)	No. per group	Noteworthy Findings	Study Number
Rabbit / New Zealand White	Intravenous (0.9% NaCl/Solution)	Gestation Day 6 through 19	5, 9	7	On Gestation Day 29, the number of litters with one or more viable fetuses was reduced for both dose groups. The % viable fetuses were reduced for both dose groups. The % post-implantation loss was elevated for both dose groups. The 5 mg/kg/day dose level clearly provided an indication of embryonic toxicity and reduced fetal viability, but the effects were less pronounced than at 9 mg/kg/day, and the fetal viability was much higher and should allow for the interpretation of rare fetal morphological changes. 5 mg/kg/day recommended as high dose for embryo-fetal development in rabbit study.	[redacted] (Non-GLP)

Confidential

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

2.6.7.12.1 ラットにおける受胎能及び着床までの初期胚発生に関する試験（試験番号 [redacted]）

Study Title: A study for effects of AR-13324 on fertility and early embryonic development up to implantation by intravenous administration in rats					
GLP	Yes	Test Article: AR-13324			
Location	4.2.3.5.1-1	Duration of Dosing	From 2 weeks pre-mating, and through the mating period until implantation (females) or the day before necropsy (males)		
Design Similar to ICH 4.1.1	Yes				
Species/Strain	Rat / Crl:CD(SD)	Day of Mating	GD0		
Initial Age	13 weeks (males), 12 weeks (females)	Day of C-section	GD15		
Date of First Dose	[redacted] 20[redacted]	Method of Administration	Intravenous		
Special Features	None	Vehicle/Formulation	0.9% NaCl/Solution		
No Observed Adverse Effect Level	General Toxicity of Parental Animals: 0.3 mg/kg/day for males and 1 mg/kg/day for females Reproductive Function of Parental Animals and Early Embryonic Development: 1 mg/kg/day				
Daily Dose (mg/kg/day)	0 (Control)	0.03	0.1	0.3	1
Males:					
No. Males	20	20	20	20	20
No. Died or Sacrificed Moribund	0	0	0	0	0
Clinical Observations					
Normal	20	20	20	20	15
Swelling, Injection site	0	0	0	0	5
Necropsy Observations					
Normal	20	19	20	20	15
Injection site: Swelling	0	0	0	0	5

GD: Gestation day

Confidential

2.6.7.12.1 ラットにおける受胎能及び着床までの初期胚発生に関する試験（試験番号 [redacted]）（続き）

Study Title: A study for effects of AR-13324 on fertility and early embryonic development up to implantation by intravenous administration in rats					
Daily Dose (mg/kg/day)	0 (Control)	0.03	0.1	0.3	1
Males:					
Testis: Small	0	1	0	0	0
Testis: Soft	0	1	0	0	0
Epididymis: Small	0	1	0	0	0
Body Weight (g) [1]	538.3	526.8 (-2.14%)	529.8 (-1.58%)	533.4 (-0.91%)	519.7 (-3.46%)
Food Consumption (g) [2]	23.8	22.1 (-7.14%)	23.6 (-0.84%)	23.6 (-0.84%)	22.4 (-5.88%)
Absolute Organ Weight (mg)					
Right Testis	1654.7	1700.2	1668.5	1673.2	1634.6
Left Testis	1664.3	1749.9	1666.1	1669.7	1629.8
Right Epididymis	663.8	643.4	661.0	675.9	642.1
Left Epididymis	648.9	657.0	637.7	655.6	634.0
Relative Organ Weight (mg/100g B.W.)					
Right Testis	308.3	324.2	315.4	314.2	315.0
Left Testis	309.9	333.1	314.9	313.6	314.3
Right Epididymis	123.4	122.8	125.1	127.0	123.9
Left Epididymis	120.7	125.0	120.6	123.1	122.4
No. Males with Successful Copulation	20	19	20	20	19

Not significantly different from control

1: At the day of necropsy (Day 45 or 46 of dosing) 2: At the day before mating period (Days 14 to 15 of dosing)

1 and 2: For controls, only group means are shown. For treated groups, in addition to group means, percent differences from controls are also shown in parentheses.

Confidential

2.6.7.12.1 ラットにおける受胎能及び着床までの初期胚発生に関する試験（試験番号 [redacted]）（続き）

Study Title: A study for effects of AR-13324 on fertility and early embryonic development up to implantation by intravenous administration in rats					
Daily Dose (mg/kg/day)	0 (Control)	0.03	0.1	0.3	1
Males:					
Copulation Rate (%)	100.0	95.0	100.0	100.0	95.0
Mean No. Copulatory Interval (day)	2.3	2.6	2.0	2.1	2.3
No. Fertile Males	20	18	17	20	18
Fertility Rate (%)	100.0	94.7	85.0	100.0	94.7
Females:					
No. Females	20	20	20	20	20
No. Died or Sacrificed Moribund	0	0	0	0	0
Clinical Observations					
Normal	20	20	20	20	20
Necropsy Observations					
Normal	20	20	20	20	20
Body Weight before Mating (g) [1]	282.7	280.6 (-0.74%)	281.9 (-0.28%)	283.4 (0.25%)	284.4 (0.60%)
Body Weight after Mating (g) [2]	359.2	361.4 (0.61%)	361.5 (0.64%)	358.8 (-0.11%)	362.2 (0.84%)
Food Consumption before Mating (g) [3]	16.6	15.6 (-6.02%)	16.8 (1.20%)	17.5 (5.42%)	17.1 (3.01%)
Food Consumption after Mating (g) [4]	24.2	24.4 (0.83%)	25.3 (4.55%)	25.2 (4.13%)	24.6 (1.65%)

Not significantly different from control

1: At the day before mating period (Day 15 of dosing), 2: At the day of necropsy (Day 15 of gestation), 3: At the day before mating period (Days 14 to 15 of dosing), 4: At the day of necropsy (Days 14 to 15 of gestation), 1 to 4: For controls, only group means are shown. For treated groups, in addition to group means, percent differences from controls are also shown in parentheses.

Confidential

2.6.7.12.1 ラットにおける受胎能及び着床までの初期胚発生に関する試験（試験番号 [redacted]）（続き）

Study Title: A study for effects of AR-13324 on fertility and early embryonic development up to implantation by intravenous administration in rats					
Daily Dose (mg/kg/day)	0 (Control)	0.03	0.1	0.3	1
Females:					
Frequency of Estrus (No. of times)	3.3	3.2	3.2	3.5	3.4
Estrous Interval (day)	4.05	4.21	4.10	4.00	4.09
No. of pairs used for mating	20	20	20	20	20
No. Females with Successful Copulation	20	19	20	20	19
Copulation Rate (%)	100.0	95.0	100.0	100.0	95.0
Mean No. Copulatory Interval (day)	2.3	2.6	2.0	2.1	2.3
No. Fertile Females	20	18	17	20	18
Fertility Rate (%)	100.0	94.7	85.0	100.0	94.7
Mean No. Corpora Lutea	15.8	14.8	15.0	14.1*	14.9
Mean No. Implantations	15.3	14.2	14.6	13.9*	14.7
Mean % Implantation	97.0	96.4	97.3	98.3	97.8
Mean % Pre-implantation Loss	3.1	3.6	2.7	1.7	2.2
Litters:					
No. Litters Evaluated	20	16	16	20	18
No. Live Embryos	294	218	223	271	245
Mean No. Live Embryos / Litter	14.7	13.6	13.9	13.6	13.6
Mean % Embryonic Viability	96.0	95.8	95.5	98.1	93.5
Mean No. Post-implantation Loss	0.6	0.6	0.6	0.3	1.1

*P<0.05: Significantly different from control

Confidential

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 ラットにおける胚・胎児発生への影響に関する試験（試験番号 [redacted]）

Study Title: Study for Effects of AR-13324 on Embryo-Fetal Development in Rats (SEG II) Following Intravenous Administration via a Vascular Access Port (VAP) (GLP)																	
GLP	Yes				Test Article: AR-13324												
Location	4.2.3.5.2-2				Duration of Dosing				12 days (GD6-GD17)								
Design Similar to ICH 4.1.3	Yes																
Species/Strain	Rat / Sprague-Dawley – Hsd:SD				Day of Mating				GD0								
Initial Age	14-15 weeks old				Day of C-section				GD20								
Date of First Dose	[redacted] 20[redacted]				Method of Administration				Intravenous								
Special Features	None				Vehicle/Formulation				0.9% NaCl/Solution								
No Observed Adverse Effect Level[1]	F0 Females: 0.1 (0.08) mg/kg/day, F1 Litters: 0.1 (0.08) mg/kg/day																
Toxicokinetics:	Netarsudil								AR-13503								
	GD6				GD17				GD6				GD17				
Daily dose (mg/kg/day)[1]	0.03 (0.022)	0.1 (0.08)	0.3	3	0.03 (0.022)	0.1 (0.08)	0.3	3	0.03 (0.022)	0.1 (0.08)	0.3	3	0.03 (0.022)	0.1 (0.08)	0.3	3	
$AUC_{(0\text{-}24hr)}$ (ng·h/mL)	0.3468	2.620	18.51	327.8	0.2358	3.368	19.86	420.0	NC	NC	0.4525	17.90	NC	NC	1.727	99.35	
$AUC_{(0\text{-}last)}$ (ng·h/mL)	0.1737	1.510	15.21	327.8	0.1181	1.838	16.12	420.0	NC	NC	0.1511	17.90	NC	NC	1.354	52.63	
$AUC_{(0\text{-}inf)}$ (ng·h/mL)	NC	NC	17.42	333.8	NC	NC	19.35	433.1	NC	NC	NC	34.44	NC	NC	NC	98.82	
C_{max} (ng/mL)	1.04	3.36	15.1	157	0.707	4.07	12.6	194	NC	NC	1.81	12.2	NC	NC	0.927	25.8	
C_{last} (ng/mL)	1.04	1.48	1.65	0.907	0.707	2.04	1.87	1.79	NC	NC	1.81	1.28	NC	NC	0.373	5.84	
C_0 (ng/mL)	1.04	5.07	25.2	226	0.707	5.76	18.1	309	NA								

NC: Not calculated, NA: Not applicable, GD: Gestation day

1: The actual doses of AR-13324 based on dose formulation analysis are shown in parentheses.

Confidential

2.6.7.13.1 ラットにおける胚・胎児発生への影響に関する試験（試験番号 [redacted]）（続き）

Study Title: Study for Effects of AR-13324 on Embryo-Fetal Development in Rats (SEG II) Following Intravenous Administration via a Vascular Access Port (VAP) (GLP)					
Daily dose (mg/kg/day)[1]	0 (Control)	0.03 (0.022)	0.1 (0.08)	0.3	3
Dam Data:					
No. Pregnant	24	24	23	24	24
No. Died/Moribund Sacrificed	0	0	0	2	0
Possible Abortions	0	0	0	9	11
Clinical Observations					
Red vaginal discharge, brown/black vaginal discharge and/or red staining on the cage tray paper	0	0	0	9	12
Mean Body Weight GD17 (g)	329.0	338.0	334.3	314.0*	295.3***
Mean Body Weight GD20 (g)	358.4	368.0	366.6	337.7	295.9***
Mean Uterus Weight (g)	69.5	80.3*	77.2	57.5	3.6###
Mean Food Consumption	-	-	-	-	-
No. with Live Fetuses	24	24	23	16	0
Litter Data:					
No. Evaluated	24	24	23	16	0
Total No. of Live Fetuses	291	346	309	211	0###

Only test article-related findings are presented in this table.

-: No noteworthy findings

GD: Gestation day, *P<0.05, ***P<0.001: Significantly different from control (Mann-Whitney U Test), ###P<0.001: Significantly different from control (Kruskal Wallis Test)

1: The actual doses of AR-13324 based on dose formulation analysis are shown in parentheses.

Confidential

2.6.7.13.1 ラットにおける胚・胎児発生への影響に関する試験（試験番号 [redacted]）（続き）

Study Title: Study for Effects of AR-13324 on Embryo-Fetal Development in Rats (SEG II) Following Intravenous Administration via a Vascular Access Port (VAP) (GLP)					
Daily dose (mg/kg/day)[1]	0 (Control)	0.03 (0.022)	0.1 (0.08)	0.3	3
Litter Data:					
No. Evaluated	24	24	23	16	0
Total No. of Dead Fetuses	0	2	1	0	0
Mean No. Corpora Lutea	-	-	-	-	-
Mean No. Implantations	-	-	-	-	-
Mean No. % Pre-Implantation Loss	-	-	-	-	-
Mean No. Early Resorptions	1.0	1.3	1.2	5.5	14.9$^{\#\#\#}$
Mean % Post-implantation Loss	8.6	8.9	8.3	38.4	100.0$^{\#\#\#}$
Male Fetal Sex Ratio	-	-	-	-	NA
Mean Fetal Body Weight (g)	3.8	3.8	3.9**	3.9***	NA
Incidence of Alterations					
Litters with Fetal Alterations[2]	9 (37.5)	9 (37.5)	6 (26.1)	5 (31.2)	NA
Fetuses with Alterations[2]	11 (3.8)	13 (3.8)	8 (2.6)	8 (3.8)	NA
% Fetuses with Alterations/Litter[3]	3.4 ± 4.9	3.6 ± 5.3	2.6 ± 5.1	3.8 ± 6.9	NA

Only test article-related findings are presented in this table.

-: No noteworthy findings

NA: Not applicable, **P<0.01, ***P<0.001: Significantly different from control (Mann-Whitney U Test), $^{\#\#\#}$P<0.001: Significantly different from control (Kruskal Wallis Test)

1: The actual doses of AR-13324 based on dose formulation analysis are shown in parentheses.

2: The total number affected per group is presented, and the percentage affected per group in presented in parentheses.

3: Group means are presented.

Confidential

2.6.7.13.1 ラットにおける胚・胎児発生への影響に関する試験（試験番号 [redacted]）（続き）

Study Title: Study for Effects of AR-13324 on Embryo-Fetal Development in Rats (SEG II) Following Intravenous Administration via a Vascular Access Port (VAP) (GLP)					
Daily dose (mg/kg/day)[1]	0 (Control)	0.03 (0.022)	0.1 (0.08)	0.3	3
Litter Data:					
No. Evaluated	24	24	23	16	0
Fetal Gross External Examination	-	-	-	-	NA
Fetal Visceral Examination	-	-	-	-	NA
Fetal Anomalies:					
Fetal Ossification Site Averages					
Mean Ossified Caudal Vertebrae	-	-	+6	+7	NA
Mean Ossified Forelimb Phalanges	-	+5	+6	+7	NA

Only test article-related findings are presented in this table.

-: No noteworthy findings

NA: Not applicable

1: The actual doses of AR-13324 based on dose formulation analysis are shown in parentheses.

Confidential

2.6.7.13.2 ウサギにおける胚・胎児発生への影響に関する試験（試験番号 [redacted]）

Study Title: Study for Effects of AR-13324 on Embryo-Fetal Development in Rabbits (SEG II) Following Intravenous Administration via a Vascular Access Port (VAP) (GLP)												
GLP	Yes			Test Article: AR-13324								
Location	4.2.3.5.2-5			Duration of Dosing			14 days (GD6-GD19)					
Design Similar to ICH 4.1.3	Yes											
Species/Strain	Rabbit / New Zealand White			Day of Mating			GD0					
Initial Age	6-7 months old			Day of C-section			GD29					
Date of First Dose	[redacted] 20[redacted]			Method of Administration			Intravenous					
Special Features	None			Vehicle/Formulation			0.9% NaCl/Solution					
No Observed Adverse Effect Level	F0 Females: 3.0 mg/kg/day, F1 Litters: 3.0 mg/kg/day											
Toxicokinetics:	Netarsudil						AR-13503					
	GD6			GD19			GD6			GD19		
Daily dose (mg/kg/day)	0.5	3.0	5.0	0.5	3.0	5.0	0.5	3.0	5.0	0.5	3.0	5.0
$AUC_{(0\text{-}24hr)}$ (ng·h/mL)	27.6	195	247	23.4	165	180	9.39	87.9	229	7.97	65.4	165
$AUC_{(0\text{-}last)}$ (ng·h/mL)	23.6	172	210	20.6	149	163	10.5[1]	77.0	179	6.37	52.2	140
$AUC_{(0\text{-}inf)}$ (ng·h/mL)	29.1	184	236	23.6	155	169	26.4[1]	82.0	205	14.4[1]	63.6	151
C_{max} (ng/mL)	26.1	73.7	150	21.4	133	148	7.11	56.2	90.1	8.79	47.5	128
C_{last} (ng/mL)	1.99	2.91	4.59	1.79	2.02	2.00	1.75	1.80	6.30	1.66	1.65	3.10
C_0 (ng/mL)	38.6	158	232	33.0	205	233	NA					

NA: Not applicable, GD: Gestation day

1: n=2

Confidential

2.6.7.13.2 ウサギにおける胚・胎児発生への影響に関する試験（試験番号 [redacted]）（続き）

Study Title: Study for Effects of AR-13324 on Embryo-Fetal Development in Rabbits (SEG II) Following Intravenous Administration via a Vascular Access Port (VAP) (GLP)				
Daily dose (mg/kg/day)	0 (Control)	0.5	3.0	5.0
Dam Data:				
No. Surviving to Day 29	19	19	18	18
No. Pregnant	14	16	17	15
Early Deaths (Test Article Unrelated)	0	0	1	1
Clinical Observations	-	-	-	-
Mean Body Weight GD29	-	-	-	-
Mean Body Weight Gain GD0-29 (kg)	0.36	0.34	0.33	0.27
Mean Fetal Weight (g)	42.06	40.44	38.25	37.98**
Mean Uterus Weight (g)	444	499	427	371
Mean Food Consumption	-	-	-	-
% with Live Fetuses	100	100	100	93
Litter Data:				
Mean No. Implantations	-	-	-	-
Mean No. Late Resorptions	-	-	-	-
% Late Resorptions	-	-	-	-
% Pre-Implantation Loss	-	-	-	-

Only test article-related findings are presented in this table.

-: No noteworthy findings

GD: Gestation day, **P<0.01: Significantly different from control (Mann-Whitney U Test)

Confidential

2.6.7.13.2 ウサギにおける胚・胎児発生への影響に関する試験（試験番号 [redacted]）（続き）

Study Title: Study for Effects of AR-13324 on Embryo-Fetal Development in Rabbits (SEG II) Following Intravenous Administration via a Vascular Access Port (VAP) (GLP)				
Daily dose (mg/kg/day)	0 (Control)	0.5	3.0	5.0
Litter Data:				
% Early Resorptions (litter incidence)	7.1	6.3	18.8	42.9
% Early Resorptions (fetal incidence)	1.2	0.9	2.4	11.9
% Viable Fetuses	98.8	96.4	94.5	87.3
% Post-Implantation Loss	1.2	3.6	5.5	12.7
Male/Female Sex Ratio	-	-	-	-
Fetal External Abnormalities	-	-	-	-
Fetal Soft Tissue Abnormalities	-	-	-	-
Fetal Skeletal Abnormalities	-	-	-	-
Fetal Ossification Abnormalities	-	-	-	-

Only test article-related findings are presented in this table.

-: No noteworthy findings

Confidential

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

2.6.7.14.1 ラットにおける出生前及び出生後の発生並びに母動物の機能に関する試験（試験番号 [redacted]）

Study Title: A study for effects of AR-13324 on pre- and postnatal development, including maternal function by intravenous administration in rats				
GLP	Yes	Test Article: AR-13324		
Location	4.2.3.5.3-1	Duration of Dosing	GD6 -PND20	
Design Similar to ICH 4.1.2	Yes			
Species/Strain	Rat / Crl:CD(SD)	Day of Mating	GD0	
Initial Age	12 to 13 weeks	Method of Administration	Intravenous	
Date of First Dose	[redacted] 20[redacted]	Vehicle/Formulation	0.9% NaCl/Solution	
Special Features	None	Litters Culled/Not culled	Culled to 4/sex/litter	
No Observed Adverse Effect Level	General Toxicity and Reproduction of Dams: 0.3 mg/kg/day, Pre- and Postnatal Development of the Next Generation: 0.3 mg/kg/day			
Daily Dose (mg/kg/day)	0 (Control)	0.03	0.1	0.3
F0 Females:				
No. Pregnant	20	20	20	20
No. Died or Sacrificed Moribund	0	0	0	0
No. Total Resorption of Litter	0	0	0	0
No. Total Litter Loss	0	0	0	0
Clinical Observations				
Gestation Period				
Normal	20	20	20	20
Lactation Period				
Normal	20	20	20	20

GD: Gestation day, PND: Postnatal day

Confidential

2.6.7.14.1 ラットにおける出生前及び出生後の発生並びに母動物の機能に関する試験（試験番号 [redacted]）（続き）

Study Title: A study for effects of AR-13324 on pre- and postnatal development, including maternal function by intravenous administration in rats				
Daily Dose (mg/kg/day)	0 (Control)	0.03	0.1	0.3
F0 Females:				
Necropsy Observations				
Normal	20	20	20	20
Gestation Body Weight (g) [1]	404.0	399.5 (-1.11%)	407.5 (0.87%)	401.8 (-0.54%)
Lactation Body Weight (g) [1]	334.5	331.4 (-0.93%)	333.2 (-0.39%)	331.9 (-0.78%)
Gestation Food Consumption (g) [1]	25.2	25.5 (1.19%)	26.1 (3.57%)	25.5 (1.19%)
Lactation Food Consumption (g) [1]	74.0	77.3 (4.46%)	77.1 (4.19%)	74.2 (0.27%)
Mean Duration of Gestation (days)	21.80	21.85	21.95	22.08
Abnormal Parturition	-	-	-	-
Delivery Index (%)	100.0	100.0	100.0	100.0

-: No noteworthy findings

Not significantly different from control

1: At end of gestation or lactation. For controls, only group means are shown. For treated groups, in addition to group means, percent differences from controls are also shown.

Confidential

2.6.7.14.1 ラットにおける出生前及び出生後の発生並びに母動物の機能に関する試験（試験番号 [redacted]）（続き）

Study Title: A study for effects of AR-13324 on pre- and postnatal development, including maternal function by intravenous administration in rats				
Daily Dose (mg/kg/day)	0 (Control)	0.03	0.1	0.3
F1 Litters (Preweaning):				
No. Litters Evaluated	20	20	20	20
Mean No. Implantations	14.9	15.2	15.3	15.1
Mean No. Pups / Litter	13.8	13.9	14.1	13.6
Mean No. Live Pups / Litter	13.6	13.7	14.1	12.9
Postnatal Survival to Day 4 after Birth (%)	96.87	96.28	97.39	95.90
Postnatal Survival to Weaning (%)	96.16	98.13	98.13	99.38
Change in Pup Body Weights (g) [1]: Male	48.42	50.65	52.54	50.58
Change in Pup Body Weights (g) [1]: Female	46.14	48.12	50.22	48.83
External Abnormalities	-	-	-	-
Pup Sex Ratios (Male/Total) [2]	0.45	0.47	0.50	0.50
Pup Clinical Signs				
Normal	20	20	20	20
Physical Development	-	-	-	-
Functional Development	-	-	-	-
Pup Necropsy Observations at Weaning				
Normal	20	20	20	19

-: No noteworthy findings

Not significantly different from control

1: From birth to weaning, 2: At birth

Confidential

2.6.7.14.1 ラットにおける出生前及び出生後の発生並びに母動物の機能に関する試験（試験番号 [redacted]）（続き）

Study Title: A study for effects of AR-13324 on pre- and postnatal development, including maternal function by intravenous administration in rats				
Daily Dose (mg/kg/day)	0 (Control)	0.03	0.1	0.3
F1 Males (Postweaning):				
No. Animals Evaluated	40	39	40	40
No. Died or Sacrificed Moribund	0	0	0	0
Clinical Observations				
Normal	40	39	40	40
Necropsy Observations				
Normal	38	39	40	40
Testis: Small	1	0	0	0
Testis: Soft	2	0	0	0
Epididymis: Small	2	0	0	0
Body Weight Change	-	-	-	-
Cleavage of the Balano-Preputial Gland	-	-	-	-
Behavioral Examination	-	-	-	-
Learning Examination	-	-	-	-
Mean Days Until Copulation	3.1	3.8	3.4	3.3
No. Males that Mated (Copulation Rate, %)	18 (90.0)	20 (100.0)	20 (100.0)	20 (100.0)
No. Fertile Males (Fertility Rate, %)	17 (94.4)	19 (95.0)	18 (90.0)	20 (100.0)

-: No noteworthy findings

Not significantly different from control

Confidential

2.6.7.14.1 ラットにおける出生前及び出生後の発生並びに母動物の機能に関する試験（試験番号 [redacted]）（続き）

Study Title: A study for effects of AR-13324 on pre- and postnatal development, including maternal function by intravenous administration in rats				
Daily Dose (mg/kg/day)	0 (Control)	0.03	0.1	0.3
F1 Females (Postweaning):				
No. Animals Evaluated	40	40	40	39
No. Died or Sacrificed Moribund	0	0	0	0
Clinical Observations				
Normal	40	40	40	39
Necropsy Observations				
Normal	40	40	40	39
Body Weight Change	-	-	-	-
Vaginal Opening	-	-	-	-
Behavioral Examination	-	-	-	-
Learning Examination	-	-	-	-
Mean Days Until Copulation	3.1	3.8	3.4	3.3
No. Females that Mated (Copulation Rate, %)	18 (90.0)	20 (100.0)	20 (100.0)	20 (100.0)
No. Fertile Females (Fertility Rate, %)	17 (94.4)	19 (95.0)	18 (90.0)	20 (100.0)

-: No noteworthy findings

Not significantly different from control

Confidential

2.6.7.14.1 ラットにおける出生前及び出生後の発生並びに母動物の機能に関する試験（試験番号 [redacted]）（続き）

Study Title: A study for effects of AR-13324 on pre- and postnatal development, including maternal function by intravenous administration in rats				
Daily Dose (mg/kg/day)	0 (Control)	0.03	0.1	0.3
F1 Dams:				
No. Pregnant	16	19	18	20
Body Weight (g)[1]	358.3	355.6 (-0.75%)	367.3 (2.51%)	355.9 (-0.67%)
Mean No. Corpora Lutea	13.3	15.3	15.4	15.3
Mean No. Implantations	13.0	13.9	14.9	14.8
Mean % Implantation	96.8	91.1	97.3	97.3
Mean % Pre-implantation Loss	3.2	8.9	2.7	2.8
F2 Litters:				
No. Litters Evaluated	16	19	18	20
Mean No. Live Embryos / Litter	12.3	13.4	13.9	13.5
Mean % Embryonic Viability	94.6	96.1	93.4	91.3
Mean No. Post-implantation Losses	0.8	0.6	1.0	1.3
Mean % Post-implantation Loss	5.4	3.9	6.6	8.8

Not significantly different from control

1: At necropsy (Day 15 of gestation). For controls, only group means are shown. For treated groups, in addition to group means, percent differences from controls are also shown.

Confidential

2.6.7.15 新生児を用いた試験

該当資料なし。

2.6.7.16 局所刺激性試験

AR-13324 の局所刺激性に関する情報は、ウサギ及びサルを用いた反復点眼毒性試験において評価できるため、独立した局所刺激性試験は実施しなかった。

2.6.7.17 その他の毒性試験

2.6.7.17.1 光安全性

Type of Study	Strain	Treatment	Doses	Noteworthy Findings	Study Number
Neutral Red Uptake Phototoxicity Assay	Balb/c 3T3 mouse fibroblasts	1 hour incubation plus UVA light for 50 min	0.490 to 30 µg/mL	According to the prediction model presented in the OECD test guidelines, AR-13324 was not predicted to have phototoxic potential (i.e., PIF < 2.0 and MPE < 0.1).	[redacted]
Neutral Red Uptake Phototoxicity Assay	Balb/c 3T3 mouse fibroblasts	1 hour incubation plus UVA, UVB light for 50 min	AR-13324: 4.64 to 1000 µg/mL[1] AR-13503: 68.1 to 1000 µg/mL[2], 6.81 to 100 µg/mL[3]	In accordance with the OECD guideline, AR-13324 (PIF of 3.2) may be considered to have probable phototoxic potential and AR-13503 (PIF >12.65) is considered to have phototoxic potential.	[redacted]

1: Range is inclusive of SSL conditions (Solar simulated light, which contains wavelengths in the visible and ultraviolet [UVA, UVB] regions) and without SSL conditions.

2: Range without SSL conditions

3: Range with SSL conditions