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2.6.7 毒性試験

2.6.7.1 毒性試験： 一覧表

Test Article: AR-13324/STN1013900 ophthalmic solution							
Type of Study Species / Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day[1])	GLP	Testing Facility	Study Number	Location in CTD
Single-Dose Toxicity							
Rat / Sprague Dawley	Intravenous	Single	1, 3, 45	No	[redacted] US	[redacted]	4.2.3.1-1
Dog / Beagle	Intravenous	Single	10, 30, 60	No	[redacted] US	[redacted]	4.2.3.1-2
Repeat-Dose Toxicity							
Rabbit / Dutch Belted	Ocular instillation	7 days	OU: 0, 24, 96, 48 µg/animal/day (0% QID, 0.04% QD and QID, 0.08% QD)	Yes[2]	[redacted] US	[redacted]	4.2.3.2-1
Rabbit / Dutch Belted	Ocular instillation	28 days	OU: 0, 24, 48, 72 µg/animal/day (0%, 0.02%, 0.04% and 0.06% BID)	Yes	[redacted] US	[redacted]	4.2.3.2-2
Rabbit / Dutch Belted	Ocular instillation	6 months	OU: 0, 14, 28, 56 µg/animal/day (0%, 0.01%, 0.02%, 0.04% BID)	Yes	[redacted] US	[redacted]	4.2.3.2-3

OU: Both eyes were treated, QD: Once daily, BID: Twice daily, QID: Four times daily

1: Unless otherwise specified

2: Toxicokinetics evaluation was conducted under non-GLP.

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2.6.7.1 毒性試験： 一覧表（続き）

Test Article: STN1013900 ophthalmic solution							
Type of Study Species / Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day[1])	GLP	Testing Facility	Study Number	Location in CTD
Rabbit / Dutch Belted	Ocular instillation	3 months	OU: 0 µg Netarsudil/ 0 µg latanoprost/animal/day BID 14 µg Netarsudil / 3.5 µg latanoprost/ animal /day QD 28 µg Netarsudil / 7 µg latanoprost/animal/day BID	Yes	[redacted] [redacted] US	[redacted]	4.2.3.2-4
Monkey / Cynomolgus	Ocular instillation	7 days	OU: 0, 24, 96, 288 µg/animal/day (0%, 0.01%, 0.04%, 0.12% QID)	Yes	[redacted] [redacted] US	[redacted]	4.2.3.2-5
Monkey / Cynomolgus	Ocular instillation	7 days	OU: 0, 24, 48, 24, 48 µg/animal/day (0%, 0.02%, 0.04% BID; 0.04%, 0.08% QD)	Yes	[redacted] [redacted] US	[redacted]	4.2.3.2-6
Monkey / Cynomolgus	Ocular instillation	35 days	OU: 0, 24, 48, 72 µg/animal/day (0%, 0.02%, 0.04%, 0.06% BID)	Yes	[redacted] [redacted] US	[redacted]	4.2.3.2-7
Monkey / Cynomolgus	Ocular instillation	9 months	OU: 0, 14, 28, 56 µg/animal/day (0%, 0.01%, 0.02%, 0.04% BID)	Yes	[redacted] [redacted] US	[redacted]	4.2.3.2-8

OU: Both eyes were treated, QD: Once daily, BID: Twice daily, QID: Four times daily

1: Unless otherwise specified

Confidential

2.6.7.1 毒性試験： 一覧表（続き）

Test Article: AR-13324							
Type of Study Species / Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day[1])	GLP	Testing Facility	Study Number	Location in CTD
Rat / Sprague Dawley	Intravenous	7 days	0, 1, 3, 10	Yes[2]	[redacted] US	[redacted]	4.2.3.2-9
Rat / Sprague Dawley	Intravenous	28 days	Intended (Protocol): 0, 0.1, 0.3, 1 Measured: 0, 0.06-0.10, 0.23-0.30, 0.84-1.06	Yes	[redacted] US	[redacted]	4.2.3.2-10
Dog / Beagle	Intravenous	7 days	0, 1, 12.5/3, 25/6	Yes[2]	[redacted] US	[redacted]	4.2.3.2-11
Dog / Beagle	Intravenous	28 days	Intended (Protocol):0, 0.1, 0.3, 1 Measured: 0, 0.06-0.07, 0.21-0.26, 0.76-0.96	Yes	[redacted] US	[redacted]	4.2.3.2-12

1: Unless otherwise specified

2: Toxicokinetics evaluation was conducted under non-GLP.

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2.6.7.1 毒性試験： 一覧表（続き）

Test Article: AR-13324							
Type of Study Species / Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day[1])	GLP	Testing Facility	Study Number	Location in CTD
Genotoxicity							
Bacterial Reverse Mutation Assay / *S. typhimurium, E. coli*	*In vitro*	NA	Initial assay: 1.5 - 5000 µg/plate Confirmatory assay: 0.20 - 60.0 µg/plate (TA98, TA100, TA1535, TA1537) and 2.0 - 600 µg/plate (WP2*uvr*A) without activation, 2.0 - 600 µg/plate with S9 activation	Yes	[redacted] [redacted] US	[redacted]	4.2.3.3.1-1
L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay	*In vitro*	NA	4-hour exposure: 0.1 - 2 µg/mL without activation, 3 - 7 µg/mL with S9 activation 24-hour exposure: 0.5 - 1.1µg/mL without activation	Yes	[redacted] [redacted] US	[redacted]	4.2.3.3.1-2
Micronucleus/ Rat, Sprague-Dawley	Intravenous	7 days	0, 1, 3, 10	Yes	[redacted] [redacted] US	[redacted]	4.2.3.2-9
Carcinogenicity – No studies conducted for this application							

NA: Not applicable

1: Unless otherwise specified

Confidential

2.6.7.1 毒性試験： 一覧表（続き）

Test Article: AR-13324							
Type of Study Species / Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day[1])	GLP	Testing Facility	Study Number	Location in CTD
Reproductive and Developmental Toxicity							
Fertility and early embryonic development, Rat / Sprague Dawley	Intravenous	From 2 weeks before mating through the mating period (males and females) until implantation (females) or the day before necropsy (males)	0, 0.03, 0.1, 0.3, 1	Yes	[redacted] [redacted] [redacted] Japan	[redacted]	4.2.3.5.1-1
Dose-finding, Rat / Sprague Dawley	Intravenous	Gestation Day 6 to 17	Intended (Protocol): 0, 0.3, 3,10 Measured: 0, 0.09, 2.4, 10	No	[redacted] [redacted] US	[redacted]	4.2.3.5.2-1
Effects on embryo-fetal development, Rat / Sprague Dawley	Intravenous	Gestation Day 6 to 17	Intended (Protocol): 0, 0.03, 0.1, 0.3, 3 Measured: 0, 0.022, 0.08, 0.3, 3	Yes	[redacted] [redacted] US	[redacted]	4.2.3.5.2-2

1: Unless otherwise specified

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2.6.7.1 毒性試験： 一覧表（続き）

Test Article: AR-13324							
Type of Study Species / Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day[1])	GLP	Testing Facility	Study Number	Location in CTD
Dose-finding, Rabbit / New Zealand White	Intravenous	Gestation Day 6 through Gestation Day 19	Intended (Protocol): 0, 0.1, 0.3, 1, 3 Measured: 0, 0.08, 0.24, 0.8, 3	No	[redacted] [redacted] US	[redacted]	4.2.3.5.2-3
Dose-finding, Rabbit / New Zealand White	Intravenous	Gestation Day 6 through Gestation Day 19	5, 9	No	[redacted] [redacted] US	[redacted]	4.2.3.5.2-4
Effects on embryo-fetal development, Rabbit / New Zealand White	Intravenous	Gestation Day 6 through Gestation Day 19	0, 0.5, 3, 5	Yes	[redacted] [redacted] US	[redacted]	4.2.3.5.2-5
Effects on pre- and postnatal development, Rat / Sprague Dawley	Intravenous	Day 6 of gestation to Day 20 after delivery	0, 0.03, 0.1, 0.3	Yes	[redacted] [redacted] [redacted] Japan	[redacted]	4.2.3.5.3-1

1: Unless otherwise specified

Confidential

2.6.7.1 毒性試験： 一覧表（続き）

Test Article: AR-13324							
Type of Study Species / Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day[1])	GLP	Testing Facility	Study Number	Location in CTD
Local Tolerance – No specific studies conducted for this application							
Other Toxicity Studies							
Neutral Red Uptake Phototoxicity Assay/ Balb/c 3T3 Mouse Fibroblasts	*In vitro*	NA	0.490 to 30 µg/mL	Yes	[redacted] US	[redacted]	4.2.3.7.7-1
Neutral Red Uptake Phototoxicity Assay/ Balb/c 3T3 Mouse Fibroblasts	*In vitro*	NA	AR-13324: 4.64 to 1000 µg/mL[2] AR-13503: 68.1 to 1000 µg/mL[3], 6.81 to 100 µg/mL[4]	Yes	[redacted] US	[redacted]	4.2.3.7.7-2

NA: Not applicable

1: Unless otherwise specified

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

3: Range without SSL conditions

4: Range with SSL conditions

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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: AR-13324/STN1013900 ophthalmic solution							
Type of Study	Species / Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day[1])	GLP	Study Number	Location in CTD
Repeat-Dose Toxicity	Rabbit / Dutch Belted	Ocular instillation	7 days	OU: 0, 24, 96, 48 µg/animal/day (0%: QID, 0.04% QD and QID, 0.08%: QD)	No	[redacted]	4.2.3.2-1
	Rabbit / Dutch Belted	Ocular instillation	28 days	OU: 0, 24, 48, 72 µg/animal/day (0%, 0.02%, 0.04% and 0.06% BID)	Yes	[redacted]	4.2.3.2-2
	Rabbit / Dutch Belted	Ocular instillation	6 months	OU: 0, 14, 28, 56 µg/animal/day (0%, 0.01%, 0.02%, 0.04% BID)	Yes	[redacted]	4.2.3.2-3
	Monkey / Cynomolgus	Ocular instillation	7 days	OU: 0, 24, 96, 288 µg/animal/day (0%, 0.01%, 0.04%, 0.12% QID)	Yes	[redacted]	4.2.3.2-5
	Monkey / Cynomolgus	Ocular instillation	7 days	OU: 0, 24, 48, 24, 48 µg/animal/day (0%, 0.02%, 0.04% BID; 0.04%, 0.08% QD)	Yes	[redacted]	4.2.3.2-6
	Monkey / Cynomolgus	Ocular instillation	35 days	OU: 0, 24, 48, 72 µg/animal/day (0%, 0.02%, 0.04%, 0.06% BID)	Yes	[redacted]	4.2.3.2-7
	Monkey / Cynomolgus	Ocular instillation	9 months	OU: 0, 14, 28, 56 µg/animal/day (0%, 0.01%, 0.02%, 0.04% BID)	Yes	[redacted]	4.2.3.2-8

OU: Both eyes were treated, QD: Once daily, BID: Twice daily, QID: Four times daily

1: Unless otherwise specified

Confidential

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（続き）

Test Article: AR-13324							
Type of Study	Species / Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day[1])	GLP	Study Number	Location in CTD
Repeat-Dose Toxicity	Rat / Sprague Dawley	Intravenous	7 days	0, 1, 3, 10	No	[redacted]	4.2.3.2-9
	Rat / Sprague Dawley	Intravenous	28 days	Intended (Protocol): 0, 0.1, 0.3, 1 Measured: 0, 0.06-0.10, 0.23-0.30, 0.84-1.06	Yes	[redacted]	4.2.3.2-10
	Dog / Beagle	Intravenous	7 days	0, 1, 12.5/3, 25/6	No	[redacted]	4.2.3.2-11
	Dog / Beagle	Intravenous	28 days	Intended (Protocol):0, 0.1, 0.3, 1 Measured: 0, 0.06-0.07, 0.21-0.26, 0.76-0.96	Yes	[redacted]	4.2.3.2-12
Reproductive and Developmental Toxicity	Rat / Sprague Dawley	Intravenous	Gestation Day 6 to 17	Intended (Protocol): 0, 0.03, 0.1, 0.3, 3 Measured: 0, 0.022, 0.08, 0.3, 3	Yes	[redacted]	4.2.3.5.2-2
	Rabbit / New Zealand White	Intravenous	Gestation Day 6 through Gestation Day 19	0, 0.5, 3, 5	Yes	[redacted]	4.2.3.5.2-5

1: Unless otherwise specified

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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

点眼投与による定常状態での血漿中ネタルスジルの C_{max} (ng/mL)

Test Article: STN1013900 ophthalmic solution				
Doses (μg/animal/day)	Rabbit		Monkey	
	Male	Female	Male	Female
24[1]	0.146	0.503	-	-
48[1]	0.526	0.441	-	-
96[1]	1.01	1.56	-	-
288[2]	-	-	0.232	0.232

C_{max} was not calculated at all dosed in Study [redacted] (Rabbit, a 28-days ocular toxicity study), Study [redacted] (Rabbit, a 6-months ocular toxicity study), Study [redacted] (Monkey, a 7-days ocular toxicity study), Study [redacted] (Monkey, a 35-days ocular toxicity study), Study [redacted] (Monkey, a 9-months ocular toxicity study).

1: Rabbit data from Study [redacted], a 7-days ocular toxicity study, Day 7

2: Monkey data from Study [redacted], a 7-days ocular toxicity study, Day 1 (C_{max} at steady state (at Day 7) was not calculated)

Confidential

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（続き）

点眼投与による定常状態での血漿中ネタルスジルの AUC (ng・h/mL)

Test Article: STN1013900 ophthalmic solution				
Doses (μg/animal/day)	Rabbit		Monkey	
	Male	Female	Male	Female
24[1]	0.2902	1.017	-	-
48[1]	3.739	0.9231	-	-
96[1]	5.348	3.499	-	-
288[2]	-	-	0.849	0.990

AUC was not calculated at all dosed in Study [redacted] (Rabbit, a 28-days ocular toxicity study), Study [redacted] (Rabbit, a 6-months ocular toxicity study), Study [redacted] (Monkey, a 7-days ocular toxicity study), Study [redacted] (Monkey, a 35-days ocular toxicity study), Study [redacted] (Monkey, a 9-months ocular toxicity study).

1: Rabbit data from Study [redacted], a 7-days ocular toxicity study, Day 7, $AUC_{0\text{-}16hr}$

2: Monkey data from Study [redacted], a 7-days ocular toxicity study, Day 1, $AUC_{0\text{-}t}$ ($AUC_{0\text{-}t}$ at steady state (at Day 7) was not calculated)

Confidential

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（続き）

点眼投与による定常状態での血漿中 AR-13503 の C_{max} (ng/mL)

すべての反復点眼試験において、ほとんどのサンプルで AR-13503 は定量下限値未満であったため、C_{max} は算出されなかった。

点眼投与による定常状態での血漿中 AR-13503 の AUC (ng・h/mL)

すべての反復点眼試験において、ほとんどのサンプルで AR-13503 は定量下限値未満であったため、AUC は算出されなかった。

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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（続き）

静脈内投与による定常状態での血漿中ネタルスジルの C_{max} (ng/mL)

Test Article: AR-13324						
Doses (mg/kg/day)[1]	Rat		Dog		Rabbit	
	Male	Female	Male	Female	Male	Female
0.03 (0.022)[2]	-	0.707	-	-	-	-
0.1 (0.06-0.07)[3]	-	-	5.56	4.76	-	-
0.1 (0.06-0.10)[4]	4.06	4.39	-	-	-	-
0.1 (0.08)[2]	-	4.07	-	-	-	-
0.3 (0.21-0.26)[3]	-	-	13.1	14.5	-	-
0.3 (0.23-0.30)[4]	18.2	12.6	-	-	-	-
0.3[2]	-	12.6	-	-	-	-
0.5[5]	-	-	-	-	-	21.4
1 (0.76-0.96)[3]	-	-	63.3	51.5	-	-
1 (0.84-1.06)[4]	64.5	57.0	-	-	-	-

1: The actual doses of AR-13324 based on dose formulation analysis are shown in parentheses.
2: Rat data from Study [redacted], an embryo-fetal development study, Gestation Day 17
3: Dog data from Study [redacted], a 28-days intravenous toxicity, Day 28
4: Rat data from Study [redacted], a 28-days intravenous toxicity, Day 28
5: Rabbit data from Study [redacted], an embryo-fetal development study, Gestation Day 19

Confidential

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（続き）

静脈内投与による定常状態での血漿中ネタルスジルの C_{max} (ng/mL)

Test Article: AR-13324						
Doses (mg/kg/day)	Rat		Dog		Rabbit	
	Male	Female	Male	Female	Male	Female
1[1]	15.1	13.6	-	-	-	-
1[2]	-	-	45.2	40.1	-	-
3[1]	61.5	46.6	-	-	-	-
3[3]	-	194	-	-	-	-
3[4]	-	-	-	-	-	133
5[4]	-	-	-	-	-	148
10[1]	182	140	-	-	-	-
12.5[2]	-	-	583	546	-	-
25[2]	-	-	1370	1477	-	-

1: Rat data from Study [redacted], a 7-days intravenous toxicity, Day 7
2: Dog data from Study [redacted], a 7-days intravenous toxicity, Day 7 (1 mg/kg/day) and Day1 (12.5 and 25 mg/kg/day)
3: Rat data from Study [redacted], an embryo-fetal development study, Gestation Day 17
4: Rabbit data from Study [redacted], an embryo-fetal development study, Gestation Day 19

Confidential

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（続き）

静脈内投与による定常状態での血漿中 AR-13503 の C_{max} (ng/mL)

Test Article: AR-13324						
Doses (mg/kg/day)[1]	Rat		Dog		Rabbit	
	Male	Female	Male	Female	Male	Female
0.3 (0.23-0.30)[2]	NC	0.950	-	-	-	-
0.3[3]	-	0.927	-	-	-	-
0.5[4]	-	-	-	-	-	8.79
1 (0.76-0.96)[5]	-	-	1.51	1.80	-	-
1 (0.84-1.06)[2]	7.00	4.10	-	-	-	-
1[6]	1.75	1.60	-	-	-	-
1[7]	-	-	1.39	1.08	-	-
3[6]	12.3	6.42	-	-	-	-
3[3]	-	25.8	-	-	-	-
3[4]	-	-	-	-	-	47.5
5[4]	-	-	-	-	-	128
10[6]	22.1	37.4	-	-	-	-
12.5[7]	-	-	30.4	11.8	-	-
25[7]	-	-	46.5	54.1	-	-

NC: Not calculated

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

2: Rat data from Study [redacted], a 28-days intravenous toxicity, Day 28

3: Rat data from Study [redacted], an embryo-fetal development study, Gestation Day 17

4: Rabbit data from Study [redacted], an embryo-fetal development study, Gestation Day 19

5: Dog data from Study [redacted], a 28-days intravenous toxicity, Day 28

6: Rat data from Study [redacted], a 7-days intravenous toxicity, Day 7

7: Dog data from Study [redacted], a 7-days intravenous toxicity, Day 7 (1 mg/kg/day) and Day1 (12.5 and 25 mg/kg/day)

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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（続き）

静脈内投与による定常状態での血漿中ネタルスジルの AUC_{0-24} (ng・h/mL)

Test Article: AR-13324						
Doses (mg/kg/day)[1]	Rat		Dog		Rabbit	
	Male	Female	Male	Female	Male	Female
0.03 (0.022)[2]	-	0.2358	-	-	-	-
0.1 (0.06-0.07)[3]	-	-	46.28	36.63	-	-
0.1 (0.06-0.10)[4]	3.6147	4.2849	-	-	-	-
0.1 (0.08)[2]	-	3.368	-	-	-	-
0.3 (0.21-0.26)[3]	-	-	54.65	108.61	-	-
0.3 (0.23-0.30)[4]	24.2798	19.4555	-	-	-	-
0.3[2]	-	19.86	-	-	-	-
0.5[5]	-	-	-	-	-	23.4
1 (0.76-0.96)[3]	-	-	481.68	334.79	-	-
1 (0.84-1.06)[4]	125.7879	121.5364	-	-	-	-

1: The actual doses of AR-13324 based on dose formulation analysis are shown in parentheses.
2: Rat data from Study [redacted], an embryo-fetal development study, Gestation Day 17
3: Dog data from Study [redacted], a 28-days intravenous toxicity, Day 28
4: Rat data from Study [redacted], a 28-days intravenous toxicity, Day 28
5: Rabbit data from Study [redacted], an embryo-fetal development study, Gestation Day 19

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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（続き）

静脈内投与による定常状態での血漿中ネタルスジルの AUC_{0-24} (ng・h/mL)

Test Article: AR-13324						
Doses (mg/kg/day)	Rat		Dog		Rabbit	
	Male	Female	Male	Female	Male	Female
1[1]	67.71	56.46	-	-	-	-
1[2]	-	-	251	278	-	-
3[1]	235.7	244.4	-	-	-	-
3[3]	-	420.0	-	-	-	-
3[4]	-	-	-	-	-	165
5[4]	-	-	-	-	-	180
10[1]	771.3	774.5	-	-	-	-
12.5[2]	-	-	3004	3379	-	-
25[2]	-	-	7021	7536	-	-

1: Rat data from Study [redacted], a 7-days intravenous toxicity, Day 7
2: Dog data from Study [redacted], a 7-days intravenous toxicity, Day 7 (1 mg/kg/day) and Day1 (12.5 and 25 mg/kg/day)
3: Rat data from Study [redacted], an embryo-fetal development study, Gestation Day 17
4: Rabbit data from Study [redacted], an embryo-fetal development study, Gestation Day 19

Confidential

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（続き）

静脈内投与による定常状態での血漿中 AR-13503 の AUC_{0-24} (ng・h/mL)

Test Article: AR-13324						
Doses (mg/kg/day)[1]	Rat		Dog		Rabbit	
	Male	Female	Male	Female	Male	Female
0.3 (0.23-0.30)[2]	NC	0.9849	-	-	-	-
0.3[3]	-	1.727	-	-	-	-
0.5[4]	-	-	-	-	-	7.97
1 (0.76-0.96)[5]	-	-	23.15	33.80	-	-
1 (0.84-1.06)[2]	4.6027	6.9281	-	-	-	-
1[6]	8.68	3.80	-	-	-	-
1[7]	-	-	18.5	4.95	-	-
3[6]	50.3	35.0	-	-	-	-
3[3]	-	99.35	-	-	-	-
3[4]	-	-	-	-	-	65.4
5[4]	-	-	-	-	-	165
10[6]	105	255	-	-	-	-
12.5[7]	-	-	275	185	-	-
25[7]	-	-	486	620	-	-

NC: Not calculated

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

2: Rat data from Study [redacted], a 28-days intravenous toxicity, Day 28

3: Rat data from Study [redacted], an embryo-fetal development study, Gestation Day 17

4: Rabbit data from Study [redacted], an embryo-fetal development study, Gestation Day 19

5: Dog data from Study [redacted], a 28-days intravenous toxicity, Day 28

6: Rat data from Study [redacted], a 7-days intravenous toxicity, Day 7

7: Dog data from Study [redacted], a 7-days intravenous toxicity, Day 7 (1 mg/kg/day) and Day1 (12.5 and 25 mg/kg/day)

Confidential

2.6.7.4 毒性試験： 被験物質（バッチ毎）一覧

2.6.7.4.1 原薬

Batch No.	Purity (%)	[redacted]	Specified Impurities (%)	Study Number	Type of Study
0428500	[redacted]	[redacted]	[redacted]	[redacted]	Acute intravenous toxicity in rats
				[redacted]	Acute intravenous toxicity in dogs
0431190	[redacted]	[redacted]		[redacted]	7-days intravenous toxicity in rats
				[redacted]	7-days intravenous toxicity in dogs
0430900	[redacted]	[redacted]		[redacted]	Bacterial reverse mutation assay
				[redacted]	*In vitro* mammalian cell gene mutation test

NA: Not applicable, [redacted]

Confidential

2.6.7.4.1 原薬（続き）

Batch No.	Purity (%)	[redacted]	Specified Impurities (%)	Study Number	Type of Study
0433385	[redacted]	[redacted]	[redacted]	[redacted]	28-days intravenous toxicity in rats
				[redacted]	28-days intravenous toxicity in dogs
0597296	[redacted]	[redacted]		[redacted]	Fertility and early embryonic development in rats
				[redacted]	Pre- and postnatal development in rats

ND: Not detected, NA: Not applicable, [redacted]

Confidential

2.6.7.4.1 原薬（続き）

Batch No.	Purity (%)	[redacted]	Specified Impurities (%) [redacted]	Study Number	Type of Study
0459933	[redacted]	[redacted]		[redacted]	Dose-finding for embryo-fetal development in rats
				[redacted]	Dose-finding for embryo-fetal development in rabbits
				[redacted]	Dose-finding for embryo-fetal development in rabbits
				[redacted]	Embryo-fetal development in rats
				[redacted]	Embryo-fetal development in rabbits
0468480	[redacted]	[redacted]		[redacted]	3T3 neutral red uptake phototoxicity assay
0476739	98.2	ND		[redacted]	3T3 neutral red uptake phototoxicity assay

ND: Not detected, NA: Not applicable, [redacted]

Confidential

2.6.7.4.2 製剤（1）

Concentration	Batch No.	Purity (%)	Specified Impurities (%)	Study Number	Type of Study
0.01%	AFY-M0036-001-01-01-0.01	[redacted]	[redacted]	[redacted]	7-days ocular toxicity in monkeys
0.04%	AFY-M0036-001-01-01-0.04	[redacted]	[redacted]	[redacted]	7-days ocular toxicity in rabbits
				[redacted]	7-days ocular toxicity in monkeys
				[redacted]	7-days ocular toxicity in monkeys
0.12%	AFY-M0036-001-01-01-0.12	[redacted]	[redacted]	[redacted]	7-days ocular toxicity in rabbits
				[redacted]	7-days ocular toxicity in monkeys
				[redacted]	7-days ocular toxicity in monkeys

BRT: Below reporting threshold, [redacted]

Confidential

2.6.7.4.2 製剤（1）（続き）

Concentration	Batch No.	Purity (%)	Specified Impurities (%)	Study Number	Type of Study
0.02%	BCL-508-149	[redacted]	[redacted]	[redacted]	28-days ocular toxicity in rabbits
				[redacted]	1-month ocular toxicity in monkeys
0.04%	BCL-508-150	[redacted]		[redacted]	28-days ocular toxicity in rabbits
				[redacted]	1-month ocular toxicity in monkeys
0.06%	BCL-508-151	[redacted]		[redacted]	28-days ocular toxicity in rabbits
				[redacted]	1-month ocular toxicity in monkeys

BRT: Below reporting threshold, [redacted]

Confidential

2.6.7.4.2 製剤（1）（続き）

Concentration	Batch No.	Purity (%)	Specified Impurities (%)	Study Number	Type of Study
0.01%	BCL-545-144B	[redacted]	[redacted]	[redacted]	6 months ocular toxicity in rabbits
				[redacted]	9-months ocular toxicity in monkeys
0.02%	BCL-545-144C	[redacted]		[redacted]	6 months ocular toxicity in rabbits
				[redacted]	9-months ocular toxicity in monkeys
0.04%	BCL-545-144D	[redacted]		[redacted]	6 months ocular toxicity in rabbits
				[redacted]	9-months ocular toxicity in monkeys

[redacted]

Confidential

2.6.7.4.3 製剤（2）

Batch No.	Purity (%)		Specified Impurities (%)	Study Number	Type of Study
	AR-13324	Latanoprost	[redacted]		
1590646	[redacted]	[redacted]		[redacted]	3 months toxicity in rabbits

BRT: Below reporting threshold, ND: Not detected, NA: Not applicable, [redacted]

[redacted]

Confidential

2.6.7.5 単回投与毒性試験

Test Article: AR-13324							
Species / Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses	Gender / No. per Group	NOAEL	Noteworthy Findings	Study Number
Rat / Sprague-Dawley	Intravenous (1, 3 mg/kg/day: 0.9% NaCl, 45 mg/kg/day: 0.9% NaCl or 4.7% Mannitol/0.05% Boric Acid/Solution)	Single	1, 3, 45 mg/kg/day	2M, 2F	3 mg/kg/day	A single iv dose of 1 and 3 mg/kg/day was well tolerated. 45 mg/kg/day was not well tolerated in either saline or borate/mannitol buffer, causing significant local irritation, including swollen and darkened tails. Additional clinical signs at 45 mg/kg/day may indicate systemic toxicity: decreased activity, hunched posture, ruffled fur coat, scant feces and scant food findings, red staining around the eyes. 45 mg/kg/day caused pink extremities, which may be related to AR-13324 pharmacology.	[redacted] (Non-GLP)
Dog / Beagle	Intravenous (10, 30 mg/kg/day: 0.9% NaCl, 60 mg/kg/day: 4.7% Mannitol/ 0.05% Boric Acid/Solution)	Single	10, 30, 60 mg/kg/day	1M, 1F	30 mg/kg/day	A single dose of 10 and 30 mg/kg/day was well tolerated. Clinical signs, decreases in body weight and food consumption, and changes in clinical pathology parameters noted at 60 mg/kg/day were consistent with acute anemia. The absence of increased reticulocytes suggests that the anemia may have been non-regenerative. Additional findings at 60 mg/kg/day that may be related to pharmacologic effects of AR-13324 included pink skin, red sclera, decreased mean blood pressure and increased heart rate.	[redacted] (Non-GLP)

NOAEL: No Observed Adverse-Effect Level

Confidential

2.6.7.6 反復投与毒性試験： 重要な試験以外の試験

Test Article: STN1013900 ophthalmic solution							
Species / Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses	Gender / No. per Group	NOAEL	Noteworthy Findings	Study Number
Rabbit / Dutch Belted	Ocular instillation OU (Vehicle for STN1013900 ophthalmic solution)	7 days + 7 days recovery	OU: 0, 24, 96, 48 µg/animal/day 0%: QID 0.04%: QD and QID 0.08%: QD	Vehicle and high dose: 8M, 8F Low and mid dose: 5M, 5F	QD: 0.08% (48 µg/animal/day) QID: <0.04% (<96 µg/animal/day)	No systemic toxicity: 0.04% and 0.08% STN1013900 ophthalmic solution (QD) treatments well tolerated, with sporadic minor ocular irritation of minimal intensity. 0.04% STN1013900 ophthalmic solution (QID) caused severe ocular irritation during the first few days of treatment but lessened in severity and incidence with continued dosing. At the end of dosing, multiple/multifocal axial punctate corneal fluorescein staining in 10 of 16 animals at 0.04% STN1013900 ophthalmic solution (QID) and 1/10 in 0.04% STN1013900 ophthalmic solution (QD) also indicated irritation but none in the 0.08% group. All eyes appeared normal by end of recovery. All dose groups evaluated microscopically; neither end of dosing nor recovery animals showed macroscopic or microscopic findings.	[redacted] (GLP)

NOAEL: No Observed Adverse-Effect Level, OU: Both eyes were treated, QD: Once daily, QID: Four times daily

Confidential

2.6.7.6 反復投与毒性試験： 重要な試験以外の試験（続き）

Test Article: STN1013900 ophthalmic solution							
Species / Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses	Gender / No. per Group	NOAEL	Noteworthy Findings	Study Number
Rabbit / Dutch Belted	Ocular instillation OU (Vehicle for STN1013900 ophthalmic solution)	28 days + 14 days recovery	OU: 0, 24, 48, 72 µg/animal/day 0%, 0.02%, 0.04%, 0.06%: BID	Vehicle and high dose: 8M, 8F Low and mid dose: 5M, 5F	0.02% BID (24 µg/animal/day)	BID dosing of 0.02% STN1013900 ophthalmic solution was generally well tolerated. Minor conjunctivitis noted sporadically during the dosing phase; few minor corneal opacities noted early in the dosing phase. Opacities had resolved by the completion of dosing, and all eyes appeared normal during the recovery phase (clinical observations and ocular observation according to Draize). Microscopic evaluation of the eyes in the 0.02% group revealed no significant findings. Animals in the 0.04% and 0.06% groups also had minor, sporadic conjunctivitis and corneal opacities that were observed during dosing phase only. Microscopic evaluation of the corneas revealed slight degeneration/erosion.	[redacted] (GLP)

NOAEL: No Observed Adverse-Effect Level, OU: Both eyes were treated, BID: Twice daily

Confidential

2.6.7.6 反復投与毒性試験： 重要な試験以外の試験（続き）

Test Article: STN1013900 ophthalmic solution							
Species / Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses	Gender / No. per Group	NOAEL	Noteworthy Findings	Study Number
Monkey / Cynomolgus	Ocular instillation OU (Vehicle for STN1013900 ophthalmic solution)	7 days + 7 days recovery	OU: 0, 24, 96, 288 µg/animal/day 0, 0.01%, 0.04%, 0.12%: QID	3M, 3F	0.01% QID (24 µg/animal/day)	0.01% STN1013900 ophthalmic solution was well tolerated. Test article-related effects were noted for administration of 0.04% and 0.12% STN1013900 ophthalmic solution. These findings consisted of gross, ophthalmologic, and histopathologic findings of inflammation, edema of the corneal stroma and/or corneal epithelium, and increased apoptosis of the corneal epithelium/endothelium (histopathologic findings persisted to the end of recovery).	[redacted] (GLP)
Monkey / Cynomolgus	Ocular instillation OU (Vehicle for STN1013900 ophthalmic solution)	7 days	OU: 0, 24, 48, 24, 48 µg/animal/day 0, 0.02%, 0.04%: BID 0.04%, 0.08%: QD	3M, 3F	0.04% BID or 0.08% QD (48 µg/animal/day)	0.02% and 0.04% STN1013900 ophthalmic solution (BID) and 0.04% and 0.08% STN1013900 ophthalmic solution (QD) were well tolerated. Gross and ophthalmologic indications of irritation noted at all dose levels were generally mild. The minimal to mild histopathologic changes were very subtle overall at all dose levels, and were not considered adverse.	[redacted] (GLP)

NOAEL: No Observed Adverse-Effect Level, OU: Both eyes were treated, QD: Once daily, BID: Twice daily, QID: Four times daily

Confidential

2.6.7.6 反復投与毒性試験： 重要な試験以外の試験（続き）

Test Article: STN1013900 ophthalmic solution/AR-13324							
Species / Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses	Gender / No. per Group	NOAEL	Noteworthy Findings	Study Number
Monkey / Cynomolgus	Ocular instillation OU (Vehicle for STN1013900 ophthalmic solution)	35 days + 14 days recovery	OU: 0, 24, 48, 72 µg/animal/day (0%, 0.02%, 0.04% 0.06%: BID)	Vehicle and high dose: 5M, 5F Low and mid dose: 3M, 3F	0.02% BID (24 µg/animal/day)	Ophthalmoscopic evidence of corneal cloudiness/haze were observed in 0.04% and 0.06% groups. Histopathologic evidence of unilateral hyperplasia of the corneal epithelium was observed in 0.06% groups, and lymphocytic infiltration of the conjunctiva and lacrimal gland were observed in all groups. Only lymphocytic infiltration of the conjunctiva still showed a slightly increased intensity in 0.06% group at the end of the recovery period.	[redacted] (GLP)
Rat / Sprague Dawley	Intravenous (0.9% NaCl/Solution)	7 days + 7-days recovery	0, 1, 3, 10 mg/kg/day	Vehicle and high dose: 15M, 15F Low and mid dose: 10M, 10F	1 mg/kg/day	No treatment related effects on body weights, food consumption and ophthalmology examinations. 1 mg/kg/day caused minor changes in clinical pathology parameters that may have been related to treatment, but not considered indicative of overt toxicity. 3 and 10 mg/kg/day 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/day group still present at end of recovery.	[redacted] (GLP)

NOAEL: No Observed Adverse-Effect Level, OU: Both eyes were treated, QD: Once daily, BID: Twice daily, QID: Four times daily

Confidential

2.6.7.6 反復投与毒性試験： 重要な試験以外の試験（続き）

Test Article: AR-13324							
Species / Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses	Gender / No. per Group	NOAEL	Noteworthy Findings	Study Number
Dog / Beagle	Intravenous (0.9% NaCl/Solution)	7 days	0, 1, 12.5 / 3, 25 / 6 mg/kg/day	3M, 3F	1 mg/kg/day	1 mg/kg/day was well tolerated. After 3 days of treatment at 12.5 and 25 mg/kg/day, several males became moribund; findings included but not limited to elevated body temperature, labored breathing, wheezing, increased respiration, dull and/or squinted eyes and decreased to no activity. 2 of 3 mid-dose males and 3 of 3 high-dose males euthanized on Day 3. Mid- and high-dose levels were reduced to 3 and 6 mg/kg/day in surviving animals. Clinical signs for all doses were forelimb/injection site edema, red skin, red eyes/sclera. 1 mg/kg/day resulted in red eyes, red skin (ears, forelimbs and abdomen), and mild to moderate swelling at the injection sites on Day 3. These signs resolved completely for the 1 mg/kg/day females on Days 7 and 8. Surviving dogs in the 25/6 mg/kg/day group had potentially adverse pathology findings in several organs (heart, kidney, lung, gallbladder), injection site alterations, and possible erythrocyte decreases.	[redacted] [redacted] (GLP)

NOAEL: No Observed Adverse-Effect Level

Confidential

2.6.7.7 反復投与毒性試験： 重要な試験

2.6.7.7.1 ウサギ６ヵ月間反復点眼試験（試験番号 [redacted]）

Study Title: A 3/6-Month Ocular Toxicity Study with AR-13324 Ophthalmic Solutions in Dutch Belted Rabbits (GLP)								
Location	4.2.3.2-3		Test Article: STN1013900 ophthalmic solution					
GLP	Yes		Duration of Dosing	3 months (interim necropsy 4 animals/sex/group) 6 months (terminal necropsy 5 animals/sex/group)				
Species/Strain	Rabbit / Dutch Belted							
Initial Age	7 months		Duration of Postdose	2 weeks (2 animals/sex groups 1 and 4)				
Method of Administration	Ocular instillation, both eyes were treated, twice daily, 35 µL/eye/dose							
Vehicle/Formulation	Vehicle for STN1013900 ophthalmic solution, 0.01%, 0.02% and 0.04% STN1013900 ophthalmic solutions							
Date of First Dose	[redacted] 20[redacted]		Special Features	None				
No Observed Adverse Effect Level	0.02% STN1013900 ophthalmic solution (28 µg/animal/day), twice daily							
Daily Dose (µg/animal/day)	Group 1 (Control) 0		Group 2 (0.01%) 14		Group 3 (0.02%) 28		Group 4 (0.04%) 56	
Number of Animals[1]	11 M	11 F	9 M	9 F	9 M	9 F	11 M	11 F
Died or Sacrificed Moribund	0	0	0	1[2]	0	0	0	0
Noteworthy Findings								
Clinical Observations								
Redness of the conjunctiva and/or sclera, Ocular discharge	+	+ to ++	+	+ to ++	+ to +++	+ to ++	+ to +++	+ to +++
Body Weight	-	-	-	-	-	-	-	-
Food Consumption	-	-	-	-	-	-	-	-

-: No noteworthy findings, +: Mild, ++: Moderate, +++: Severe

1: Two male and 2 female animals in the Group 1 and 4 were evaluated as the recovery group., 2: Euthanized for humane reasons (broken forelimb)

Confidential

2.6.7.7.1 ウサギ６ヵ月間反復点眼試験（試験番号 [redacted]）（続き）

Study Title: A 3/6-Month Ocular Toxicity Study with AR-13324 Ophthalmic Solutions in Dutch Belted Rabbits (GLP)								
Daily Dose (μg/animal/day)	Group 1 (Control) 0		Group 2 (0.01%) 14		Group 3 (0.02%) 28		Group 4 (0.04%) 56	
Number of Animals[1]	11 M	11 F	9 M	9 F	9 M	9 F	11 M	11 F
Noteworthy Findings								
Ocular Observations[2]								
Conjunctival irritation	-	-	-	-	+	+	+	+ to +++
Ophthalmology[3]								
Conjunctival irritation	-	-	-	+	+	+ to ++	+	+
Electroretinography	-	-	-	-	-	-	-	-
Intraocular Pressure								
Mean intraocular pressure	-	-	-	A	A	-	A	-
Hematology	-	-	-	-	-	-	-	-
Serum Chemistry	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-
Organ Weights	-	-	-	-	-	-	-	-

-: No noteworthy findings, +: Mild, ++: Moderate, +++: Severe, A: Decrease

1: Two male and 2 female animals in the Group 1 and 4 were evaluated as the recovery group.

2: Draize scoring method

3: McDonald and Shadduck scoring

Confidential

2.6.7.7.1 ウサギ６ヵ月間反復点眼試験（試験番号 [redacted]）（続き）

Study Title: A 3/6-Month Ocular Toxicity Study with AR-13324 Ophthalmic Solutions in Dutch Belted Rabbits (GLP)								
Daily Dose (μg/animal/day)	Group 1 (Control) 0		Group 2 (0.01%) 14		Group 3 (0.02%) 28		Group 4 (0.04%) 56	
Number of Animals[1]	11 M	11 F	9 M	9 F	9 M	9 F	11 M	11 F
Histopathology								
Attenuation, corneal epithelium	-	-	-	-	-	-	+ to ++	-
Inflammation, mixed cell, cornea, peripheral	-	-	-	-	-	-	+	+
Ulcer, corneal epithelium	-	-	-	-	-	-	+	-
Vascularization, cornea, peripheral	-	-	-	-	-	-	+	-
Postdose Evaluation	-	-					-	-
Plasma Concentrations (ng/mL)								
Netarsudil	BLQ	BLQ	NA	NA	BLQ	BLQ	2.09[2]	BLQ
AR-13503	BLQ	BLQ	NA	NA	1.51[2]	1.05[2]	1.03-1.53[3]	1.02-1.60[4]

BLQ: Below the lower limit of quantification (<1.00 ng/mL), NA: Not applicable

1: Two male and 2 female animals in the Group 1 and 4 were evaluated as the recovery group.

2: n=1

3: Plasma concentration range of 4 animals

4: Plasma concentration range of 6 animals

Confidential

2.6.7.7.2 ウサギ 3 ヵ月間反復点眼毒性試験（PG324 点眼液）（試験番号 [redacted]）

Study Title: A 3-Month Ocular Toxicity Study with PG324 Ophthalmic Solution in Dutch Belted Rabbits (GLP)						
Location	4.2.3.2-4		Test Article: PG324 ophthalmic solution			
GLP	Yes		Duration of Dosing	3 months		
Species/Strain	Rabbit / Dutch Belted		Duration of Postdose	2 weeks (2 animals/sex/group)		
Initial Age	5-10 months		Date of First Dose	[redacted] 20[redacted]		
Method of Administration	Ocular instillation, both eyes were treated, Groups 1 and 3: twice daily, Group 2: once daily, 35 µL/eye/dose					
Vehicle/Formulation	Vehicle for STN1013900 ophthalmic solution, PG324 ophthalmic solution (Netarsudil 0.02% and Latanoprost 0.005%)					
Special Features	None					
No Observed Adverse Effect Level	PG324 ophthalmic solution (Netarsudil 0.02% and Latanoprost 0.005%), twice daily					
Daily Dose (µg/animal/day) (Netarsudil/Latanoprost)	Group 1 (Control) 0 / 0		Group 2 14 / 3.5		Group 3 28 / 7	
Number of Animals[1]	7 M	7 F	7 M	7 F	7 M	7 F
Died or Sacrificed Moribund	0	0	0	0	0	0
Noteworthy Findings						
Clinical Observations						
Redness of the eyes (conjunctivae and/or sclera)	+ to ++	-	+ to ++	+ to ++	+ to ++	+ to ++
Ocular discharge	+	-	-	-	-	+
Body Weight	-	-	-	-	-	-
Food Consumption	-	-	-	-	-	-

-: No noteworthy findings, +: Mild, ++: Moderate, +++: Severe

1: Two male and 2 female animals in the all groups were evaluated as the recovery group.

Confidential

2.6.7.7.2 ウサギ3ヵ月間反復点眼毒性試験（PG324 点眼液）（試験番号 [redacted]）（続き）

Study Title: A 3-Month Ocular Toxicity Study with PG324 Ophthalmic Solution in Dutch Belted Rabbits (GLP)						
Daily Dose (μg/animal/day)	Group 1 (Control) 0 / 0		Group 2 14 / 3.5		Group 3 28 / 7	
Number of Animals[1]	7 M	7 F	7 M	7 F	7 M	7 F
Noteworthy Findings						
Ocular Observations						
Conjunctival redness	+	-	+ to ++	+ to ++	+	+
Ophthalmology						
Conjunctival discharge	-	-	-	-	-	+
Electroretinography	-	-	-	-	-	-
Intraocular Pressure						
Mean intraocular pressure	-	-	-	A	A	-
Hematology	-	-	-	-	-	-
Serum Chemistry	-	-	-	-	-	-
Organ Weights	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-
Postdose Evaluation						
Clinical Observations						
Conjunctival redness	-	-	+	-	-	-

-: No noteworthy findings, +: Mild, ++: Moderate, +++: Severe, A: Decrease

1: Two male and 2 female animals in the all groups were evaluated as the recovery group.

Confidential

2.6.7.7.3 サル９ヵ月間反復点眼毒性試験（試験番号 [redacted]）

Study Title: A 9-Month GLP Ocular Toxicity Study of AR-13324 Ophthalmic Solutions following Topical Ocular Administration in Cynomolgus Monkeys with a 4-Week Recovery Phase								
Location	4.2.3.2-8		Test Article: STN1013900 ophthalmic solution					
GLP	Yes		Duration of Dosing		97 days - interim necropsy 4M/4F groups 1, 3, 4; 3M/3F group 2 272 days - main study 4M/4F groups 1, 3, 4; 3M/3F group 2			
Species/Strain	Monkey / Cynomolgus		Duration of Postdose		4 weeks (2 animals/sex/groups 1,3,4)			
Initial Age	2.4-3.2 years		Method of Administration		Ocular instillation, both eyes were treated, twice daily, 35 µL/eye/dose			
Date of First Dose	[redacted] 20[redacted]		Special Features		None			
Vehicle/Formulation	Vehicle for STN1013900 ophthalmic solution, 0.01%, 0.02% and 0.04% STN1013900 ophthalmic solutions							
No Observed Adverse Effect Level	0.04% STN1013900 ophthalmic solution (56 µg/animal/day), twice daily							
Daily Dose (µg/animal/day)	Group 1 (Control) 0		Group 2 (0.01%) 14		Group 3 (0.02%) 28		Group 4 (0.04%) 56	
Number of Animals[1]	10 M	10 F	6 M	6 F	10 M	10 F	10 M	10 F
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Noteworthy Findings								
Clinical Observations								
Eyelid reddening[2]	-	-	-	-	-	-	X	X
Partial eye closure[2]	-	-	-	-	X	-	X	X
Body Weight	-	-	-	-	-	-	-	-
Food Consumption	-	-	-	-	-	-	-	-

-: No noteworthy findings, +: Mild, ++: Moderate, +++: Severe, X: Present

1: Two male and 2 female animals in the Group 1, 3 and 4 were evaluated as the recovery group.

2: Severity was not recorded.

Confidential

2.6.7.7.3 サル 9 ヵ月間反復点眼毒性試験（試験番号 [redacted]）（続き）

Study Title: A 9-Month GLP Ocular Toxicity Study of AR-13324 Ophthalmic Solutions following Topical Ocular Administration in Cynomolgus Monkeys with a 4-Week Recovery Phase								
Daily Dose (μg/animal/day)	Group 1 (Control) 0		Group 2 (0.01%) 14		Group 3 (0.02%) 28		Group 4 (0.04%) 56	
Number of Animals[1]	10 M	10 F	6 M	6 F	10 M	10 F	10 M	10 F
Noteworthy Findings								
Ocular Observations								
Hyperemia, chemosis, or discharge	-	+	+	-	+	+	+ to ++	+
Ophthalmology								
Corneal haze	-	-	-	-	-	+	+	+
Indirect Ophthalmoscopy	-	-	-	-	-	-	-	-
Electroretinography	-	-	-	-	-	-	-	-
Intraocular Pressure								
Mean intraocular pressure	-	-	A	A	A	A	A	A
Hematology	-	-	-	-	-	-	-	-
Serum Chemistry	-	-	-	-	-	-	-	-
Organ Weights	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-	-	-
Postdose Evaluation	-	-			-	-	-	-

-: No noteworthy findings, +: Mild, ++: Moderate, +++: Severe, A: Decrease

1: Two male and 2 female animals in the Group 1, 3 and 4 were evaluated as the recovery group.

Confidential

2.6.7.7.3 サル９ヵ月間反復点眼毒性試験（試験番号 [redacted]）（続き）

Study Title: A 9-Month GLP Ocular Toxicity Study of AR-13324 Ophthalmic Solutions following Topical Ocular Administration in Cynomolgus Monkeys with a 4-Week Recovery Phase								
Daily Dose (μg/animal/day)	Group 1 (Control) 0		Group 2 (0.01%) 14		Group 3 (0.02%) 28		Group 4 (0.04%) 56	
Number of Animals[1]	10 M	10 F	6 M	6 F	10 M	10 F	10 M	10 F
Plasma Concentrations (ng/mL)								
Netarsudil	BLQ	BLQ	NA	NA	BLQ	BLQ	BLQ	BLQ
AR-13503	BLQ	BLQ	NA	NA	BLQ	BLQ	BLQ	BLQ

-: No noteworthy findings, +: Mild, ++: Moderate, +++: Severe

NA: Not applicable, BLQ: Below the lower limit of quantification (<1.00 ng/mL)

1: Two male and 2 female animals in the Group 1, 3 and 4 were evaluated as the recovery group.

Confidential

2.6.7.7.4 ラット 28 日間反復静脈内投与試験（試験番号 [redacted]）

Study Title: A 28-Day GLP Intravenous Toxicity Study of AR-13324 in Rats with a 14-Day Recovery								
Location	4.2.3.2-10		Test Article: AR-13324					
GLP	Yes		Duration of Dosing		28 days			
Species/Strain	Rat / Sprague Dawley		Duration of Postdose		14 days (5 animals/sex groups 1 and 4)			
Initial Age	7 weeks		Method of Administration		Intravenous, once daily			
Date of First Dose	[redacted] 20[redacted] (Males) [redacted] 20[redacted] (Females)		Vehicle/Formulation		0.9% NaCl/Solution			
Special Features	None							
No Observed Adverse Effect Level[1]	1 (0.84-1.06) mg/kg/day							
Toxicology Group No.	Group 1 (Control)		Group 2		Group 3		Group 4	
Daily Dose (mg/kg/day)[1]	0		0.1 (0.06-0.1)		0.3 (0.23-0.3)		1 (0.84-1.06)	
Number of Animals[2]	15 M	15 F	10 M	10 F	10 M	10 F	15 M	15 F
Died or Sacrificed Moribund[3]	2	2	0	1	0	0	0	0
Noteworthy Findings								
Clinical Observations	-	-	-	-	-	-	-	-
Body Weight	-	-	-	-	-	-	-	-
Food Consumption	-	-	-	-	-	-	-	-
Ophthalmology	-	-	-	-	-	-	-	-

-: No noteworthy findings, +: Mild, ++: Moderate, +++: Severe

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

2: Five male and 5 female animals in the Group 1 and 4 were evaluated as the recovery group.

3: All deaths were related to dosing procedures.

Confidential

2.6.7.7.4 ラット 28 日間反復静脈内投与試験（試験番号 [redacted]）（続き）

Study Title: A 28-Day GLP Intravenous Toxicity Study of AR-13324 in Rats with a 14-Day Recovery								
Toxicology Group No.	Group 1 (Control)		Group 2		Group 3		Group 4	
Daily Dose (mg/kg/day)[1]	0		0.1 (0.06-0.1)		0.3 (0.23-0.3)		1 (0.84-1.06)	
Number of Animals[2]	15 M	15 F	10 M	10 F	10 M	10 F	15 M	15 F
Noteworthy Findings								
Hematology	-	-	-	-	-	-	-	-
Serum Chemistry	-	-	-	-	-	-	-	-
Organ Weights	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-	-	-
Postdose Evaluation	-	-					-	-

-: No noteworthy findings, +: Mild, ++: Moderate, +++: Severe

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

2: Five male and 5 female animals in the Group 1 and 4 were evaluated as the recovery group.

Confidential

2.6.7.7.4 ラット 28 日間反復静脈内投与試験（試験番号 [redacted]）（続き）

Study Title: A 28-Day GLP Intravenous Toxicity Study of AR-13324 in Rats with a 14-Day Recovery						
Toxicokinetics Group No.	Group 5		Group 6		Group 7	
Daily Dose (mg/kg/day)[1]	0.1 (0.06-0.1)		0.3 (0.23-0.3)		1 (0.84-1.06)	
Number of Animals	9 M	9 F	9 M	9 F	9 M	9 F
Died or Sacrificed Moribund[2]	1	0	0	0	0	1
TK parameter for Netarsudil (Day 1/ Day 28)						
AUC_{0-t} (ng·h/mL)	4.5982 / 1.8522	4.4868 / 3.9119	30.8279 / 20.7398	19.9858 / 16.8155	103.0820 / 101.8679	104.7314 / 91.8564
AUC_{0-24h} (ng·h/mL)	4.9412 / 3.6147	4.8268 / 4.2849	37.5239 / 24.2798	23.0258 / 19.4555	130.8420 / 125.7879	138.6514 / 121.5364
T_{max} (h)	0.167 / 0.167	0.167 / 0.167	0.167 / 0.167	0.167 / 0.167	0.167 / 0.167	0.167 / 0.167
C_{max} (ng/mL)	4.74 / 4.06	4.39 / 4.39	19.7 / 18.2	9.80 / 12.6	64.9 / 64.5	59.4 / 57.0
TK parameter for AR-13503 (Day 1/ Day 28)						
AUC_{0-t} (ng·h/mL)	NC / NC	NC / NC	0.0718 / NC	0.333 / 0.2724	3.6233 / 4.2597	2.1649 / 6.9281
AUC_{0-24h} (ng·h/mL)	NC / NC	NC / NC	0.2150 / NC	0.7083 / 0.9849	5.2033 / 4.6027	6.2524 / 6.9281
T_{max} (h)	NC / NC	NC / NC	0.167 / NC	0.167 / 0.5	0.5 / 0.167	0.5 / 0.167
C_{max} (ng/mL)	NC / NC	NC / NC	0.860 / NC	1.00 / 0.950	1.99 / 7.00	5.45 / 4.10

Each TK parameter was calculated using average concentration.

NC: Not calculated

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

2: All deaths were related to dosing procedures.

Confidential

2.6.7.7.5 イヌ 28 日間反復静脈内投与試験（試験番号 [redacted]）

Study Title: 28-Day GLP Intravenous Toxicity Study of AR-13324 in Beagle Dogs with a 2-Week Recovery								
Location	4.2.3.2-12				Test Article: AR-13324			
GLP	Yes				Duration of Dosing		28 days	
Species/Strain	Dog / Beagle				Duration of Postdose		14 days (2 animals/groups 1, 4)	
Initial Age	5 months				Method of Administration		Intravenous, once daily	
Date of First Dose	[redacted] 20[redacted] (Males) [redacted] 20[redacted] (Females)				Vehicle/Formulation		0.9% NaCl/Solution	
No Observed Adverse Effect Level[1]	1 (0.76-0.96) mg/kg/day				Special Features		None	
Daily Dose (mg/kg/day)[1]	Group 1 (Control) 0		Group 2 0.1 (0.06-0.07)		Group 3 0.3 (0.21-0.26)		Group 4 1 (0.76-0.96)	
Number of Animals[2]	5 M	5 F	3 M	3 F	3 M	3 F	5 M	5 F
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Noteworthy Findings								
Clinical Observations								
Injection site swelling	-	-	+	+	+	+	+, +++	+, +++
Body Weight	-	-	-	-	-	-	-	-
Food Consumption	-	-	-	-	-	-	-	-
Ophthalmology	-	-	-	-	-	-	-	-

-: No noteworthy findings, +: Mild, ++: Moderate, +++: Severe

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

2: Two male and 2 female animals in the Group 1 and 4 were evaluated as the recovery group.

Confidential

2.6.7.7.5 イヌ 28 日間反復静脈内投与試験（試験番号 [redacted]）（続き）

Study Title: 28-Day GLP Intravenous Toxicity Study of AR-13324 in Beagle Dogs with a 2-Week Recovery								
Daily Dose (mg/kg/day)[1]	Group 1 (Control) 0		Group 2 0.1 (0.06-0.07)		Group 3 0.3 (0.21-0.26)		Group 4 1 (0.76-0.96)	
Number of Animals[2]	5 M	5 F	3 M	3 F	3 M	3 F	5 M	5 F
Noteworthy Findings								
Intraocular Pressure	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Serum Chemistry	-	-	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-	-	-
Organ Weights	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-	-	-
Postdose Evaluation	-	-	-	-	-	-	-	-

-: No noteworthy findings, +: Mild, ++: Moderate, +++: Severe

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

2: Two male and 2 female animals in the Group 1 and 4 were evaluated as the recovery group.

Confidential

2.6.7.7.5 イヌ 28 日間反復静脈内投与試験（試験番号 [redacted]）（続き）

Study Title: 28-Day GLP Intravenous Toxicity Study of AR-13324 in Beagle Dogs with a 2-Week Recovery									
Daily Dose (mg/kg/day)[1]		Group 1 (Control) 0		Group 2 0.1 (0.06-0.07)		Group 3 0.3 (0.21-0.26)		Group 4 1 (0.76-0.96)	
Number of Animals[2]		5 M	5 F	3 M	3 F	3 M	3 F	5 M	5 F
TK parameter for Netarsudil									
AUC_{0-t} (ng·h/mL)	Day 1	NC	NC	10.11±3.177	11.15±4.693	29.57±1.755	57.60±19.999	281.61±62.025	209.18±43.359
	Day 7	NC	NC	39.33±20.581	31.55±20.784	49.54±23.263	110.99±34.763	426.88±97.210	309.38±83.903
	Day 28	NC	NC	42.10±20.241	28.21±20.616	34.31±4.081	108.61±27.838	481.68±151.932	334.79±96.125
AUC_{0-24h} (ng·h/mL)	Day 1	NC	NC	17.31±7.620	16.16±9.259	47.49±6.127	65.47±6.736	281.61±62.025	209.18±43.359
	Day 7	NC	NC	42.71±14.766	41.07±12.923	59.78±15.274	110.99±34.763	426.88±97.210	309.38±83.903
	Day 28	NC	NC	46.28±13.003	36.63±13.463	54.65±11.099	108.61±27.838	481.68±151.932	334.79±96.125
T_{max} (h)	Day 1	NC	NC	0.167±0	0.167±0	0.167±0	0.167±0	0.167±0	0.167±0
	Day 7	NC	NC	0.167±0	0.389±0.1923	0.167±0	0.167±0	0.167±0	0.167±0
	Day 28	NC	NC	0.167±0	0.167±0	0.167±0	0.167±0	0.167±0	0.167±0
C_{max} (ng/mL)	Day 1	NC	NC	3.18±0.287	4.56±0.555	11.4±2.20	11.8±0.12	52.7±11.21	41.8±3.42
	Day 7	NC	NC	4.96±0.724	5.60±0.185	13.2±1.29	14.1±2.14	67.8±6.53	51.3±6.74
	Day 28	NC	NC	5.56±0.609	4.76±0.650	13.1±0.36	14.5±1.46	63.3±11.17	51.5±4.41

Each TK parameter was calculated using average concentration.

NC: Not calculated

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

2: Two male and 2 female animals in the Group 1 and 4 were evaluated as the recovery group.

Confidential

2.6.7.7.5 イヌ 28 日間反復静脈内投与試験（試験番号 [redacted]）（続き）

Study Title: 28-Day GLP Intravenous Toxicity Study of AR-13324 in Beagle Dogs with a 2-Week Recovery									
Daily Dose (mg/kg/day)[1]		Group 1 (Control) 0		Group 2 0.1 (0.06-0.07)		Group 3 0.3 (0.21-0.26)		Group 4 1 (0.76-0.96)	
Number of Animals[2]		5 M	5 F	3 M	3 F	3 M	3 F	5 M	5 F
TK parameter for AR-13503									
AUC_{0-t} (ng·h/mL)	Day 1	NC	NC	NC	NC	NC	NC	NC	NC
	Day 7	NC	NC	NC	NC	NC	NC	7.81[3]	12.62±9.635
	Day 28	NC	NC	NC	NC	NC	NC	14.25±11.572	31.51±12.791
AUC_{0-24h} (ng·h/mL)	Day 1	NC	NC	NC	NC	NC	NC	NC	NC
	Day 7	NC	NC	NC	NC	NC	NC	16.21[3]	20.97±5.153
	Day 28	NC	NC	NC	NC	NC	NC	23.15±6.884	33.80±8.086
T_{max} (h)	Day 1	NC	NC	NC	NC	NC	NC	NC	NC
	Day 7	NC	NC	NC	NC	NC	NC	2.1[3]	3.7±4.03
	Day 28	NC	NC	NC	NC	NC	NC	6.4±2.19	6.8±2.68
C_{max} (ng/mL)	Day 1	NC	NC	NC	NC	NC	NC	NC	NC
	Day 7	NC	NC	NC	NC	NC	NC	1.26[3]	1.50±0.223
	Day 28	NC	NC	NC	NC	NC	NC	1.51±0.201	1.80±0.288

Each TK parameter was calculated using average concentration.

NC: Not calculated

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

2: Two male and 2 female animals in the Group 1 and 4 were evaluated as the recovery group.

3: n=2

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