CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208254Orig1s000

NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	208254
Supporting document/s:	1: Original NDA
	7: NDA resubmission after withdrawal
	10: Labeling/Package insert draft
Applicant's letter date:	SD # 1: 8-30-2016
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	SD # 10: 5-11-2017
CDER stamp date:	SD # 1: 8-30-2016
	SD # 7: 2-28-2017
	SD # 10: 5-11-2017
Product:	Rhopressa™ (netarsudil ophthalmic solution) 0.02%
Indication:	Reduction of elevated intraocular pressure in patients with open-angle glaucoma or ocular hypertension
Applicant:	Aerie Pharmaceuticals, Inc
Review Division:	Transplant and Ophthalmology Products
Reviewer:	Maria I Rivera, PhD
Supervisor/Team Leader:	Lori E. Kotch, PhD
Division Director:	Renata Albrecht, MD
Project Manager:	Eithu Lwin

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1 Executive Summary

1.1 Introduction

Netarsudil mesylate (AR-13324) is a new chemical entity Rho-associated protein kinase inhibitor (ROCK) being developed as a topical formulation (RHOPRESSA, Netarsudil Ophthalmic Solution 0.02%) for the indication of reduction of intraocular pressure (IOP) in patients with open-angle glaucoma or ocular hypertension. The proposed dosing regimen is one drop once daily in the evening.

Netarsudil mesylate is a potent inhibitor for both isoforms of human Rho kinase (ROCK1 and ROCK2), with activity in the low nanomolar range in Rho-kinase enzymatic assays. AR-13503, the main active metabolite of netarsudil produced by esterase hydrolysis, has 5-fold greater *in vitro* activity against both Rho kinases compared to netarsudil. Netarsudil's efficacy as an IOP lowering agent appears to occur by multiple mechanisms: increasing outflow through the trabecular meshwork, decreasing aqueous humor production, and decreasing episcleral venous pressure. This combination of mechanisms is unique for an ocular hypotensive medication, as most currently used glaucoma medications reduce IOP by either reducing the production of aqueous humor or by improving the drainage of this fluid through the non-conventional uveoscleral pathway.

The applicant claims that netarsudil mesylate is also a (b) (4)

(b) (4)

1.2 Brief Discussion of Nonclinical Findings

Nonclinical studies support (b) (4) section 12.1 Mechanism of Action of the proposed label: increasing trabecular outflow facility,

(b) (4)

(b) (4)

The IC_{50} for the inhibitory effect of AR-13324 on hERG potassium current was 0.4 µM (181 ng/mL). QTc prolongation was not observed in the safety pharmacology study in dogs at doses (based on free base) up to 17.6 mg/kg IV (872 ng/mL) or a 28-day repeat-dose toxicity study in dogs at doses up to 0.53-0.67 mg/kg (51-63 ng/mL). As noted by

the applicant, only 3% of netarsudil is expected to be unbound to plasma protein *in vivo*. Therefore, low levels of active free drug would explain the lack of QTc prolongation in vivo. Clinical exposure of netarsudil was generally below the lower limit of quantitation of 0.100 ng/mL at the recommended clinical dose, indicating minimal risk for QTc prolongation to be observed in humans at the intended dosing regimen.

The ocular safety of Netarsudil Ophthalmic Solution was evaluated in rabbits and monkeys in repeat-dose ocular toxicity studies of 7-day, 28-day, 3/6-month (rabbits) and 9-month (monkey) duration. Findings in both species included signs of ocular irritation, (i.e., conjunctival congestion [hyperemia], discharge and/or swelling), corneal alterations (haze, opacities, peripheral vascularization, mixed cell inflammation, attenuation of the overlying corneal epithelium, punctate ulcers, hypertrophy/hyperplasia, edema, and/or apoptosis), and decreased IOP (intended pharmacological effect). The severity and extent of these findings was dose dependent.

Generally, the signs of ocular irritation and corneal alterations decreased in severity or resolved despite continuous dosing, or resolved during the recovery period. The applicant stated that findings of conjunctival hyperemia, conjunctival hemorrhage, and erythema of the eyelids were observed in the Phase 3 studies. The conjunctival hyperemia could be related to the pharmacological effect of dilatation of blood vessels. This reviewer agrees with the applicant that conjunctival hyperemia (without signs of ocular irritation) is not a toxicological concern. Exposure margins were 8-fold (monkey) and 2-fold (rabbit) at the intended clinical dose regimen, based on the NOAEL from the chronic ocular toxicity studies.

The NOEL for corneal haze provides an exposure margin of 1 at the intended clinical dose. Netarsudil is a cationic amphiphilic drug, which are known to induce phospholipidosis. Netarsudil induced phospholipidosis in a cell-based assay. As noted by the applicant, it is possible that the finding of corneal haze in monkeys is related to the findings of “corneal deposits” and “corneal verticillata” associated with Netarsudil Ophthalmic Solution 0.02% treatment in Phase 3 clinical studies. The applicant indicated that an observational clinical study of subjects who developed corneal deposits in the pivotal Phase 3 trials is being conducted to assess changes in corneal deposits over time and effects in visual function. The applicant plans to include the results in the NDA 4-month safety update. As the corneal haze resolved in monkeys despite continued dosing (all but one animal), or resolved during the recovery period (one animal), this proposal is considered acceptable from the nonclinical perspective.

The systemic toxicity profile of netarsudil was evaluated in rats and dogs in repeat-dose studies of 7-day and 28-day duration by the intravenous route. Based on the results of the systemic toxicity studies in rats and dogs, as well as the cardiovascular safety pharmacology study in telemetered dogs, common findings included cardiovascular (decreased blood pressure, increased heart rate) effects and inflammation at the site of injection. The vasodilation is an expected pharmacological effect of Rho-kinase inhibitors. Additional targets observed at the highest doses tested in the early short-term (7-day) repeat-dose IV toxicity studies in rats and/or dogs included the hematopoietic system

(RBC parameters and WBC types alterations, bone marrow and lymph nodes microscopic findings), blood coagulation system (increased PT and/or APTT) and several organs (microscopic findings in the heart, kidneys, lungs, gallbladder, urinary bladder and thymus). Based on the observed clinical exposure of netarsudil generally below the lower limit of quantitation of 0.100 ng/mL at the recommended clinical dose, it is unlikely these findings are clinically relevant.

Embryofetal development toxicity studies in both rats and rabbits showed dose dependent increases in parameters associated with toxicity to the embryo and fetus such as increased early resorptions, increased in post-implantation loss, decreases in litter size, decreased number of mothers with viable fetuses, and decreased number of viable fetuses.

Signs indicative of abortions were observed in rats. Offspring anomalies were limited to a slight increase in the mean number of ossified caudal vertebrae at ≥0.1 mg/kg/day and forelimb phalanges at ≥0.03 mg/kg/day.

The applicant concluded that there were no AR-13324-related fetal external, soft tissue, or skeletal fetal malformations or variations at any dose in rabbits. However, there were some findings with higher incidence in test-article treated groups. These included umbilical hernia and thoracogastroschisis in one high-dose (5.0 mg/kg/day) and one mid-dose (3.0 mg/kg/day) fetus, respectively, and increased number of fetuses with absent intermediate lung lobe at 5.0 mg/kg/day. Although the umbilical hernia and thoracogastroschisis were observed in one fetus each, these are rare findings. In addition, the incidence was above the historical control range (Charles River Historical Control database, June 2008-2010).

The exposure margins at the NOAEL for embryofetal development toxicity were >40-fold (rat) and >1300-fold (rabbit). Based on the observed clinical exposure of netarsudil generally below the lower limit of quantitation of 0.100 ng/mL at the recommended clinical dose, there is low risk for embryofetal toxicity to be observed at the intended dosing regimen.

Netarsudil did not show mutagenic or clastogenic potential in the full battery of genetic toxicity studies conducted. Carcinogenicity studies were not conducted. The justification provided by the applicant to omit these studies was considered acceptable (see Section 8. Carcinogenicity).

In conclusion, the nonclinical data presented in this NDA provides adequate safety support for the intended dosing regimen of Netarsudil Ophthalmic Solution 0.02% once daily ((b) (4) mg/eye) for the indication of reduction of IOP.

1.3 Recommendations

1.3.1 Approvability

Approval is recommended from the nonclinical perspective.

1.3.2 Additional Nonclinical Recommendations

None

1.3.3 Labeling

Applicant's proposed text	Reviewer's revisions[1]
INDICATIONS AND USAGE RHOPRESSA™ (netarsudil ophthalmic solution) 0.02% is a Rho kinase (b) (4) inhibitor indicated for the reduction of elevated intraocular pressure in patients with open angle glaucoma or ocular hypertension.	**INDICATIONS AND USAGE** RHOPRESSA™ (netarsudil ophthalmic solution) 0.02% is a Rho-*associated protein* kinase (b) (4) -inhibitor indicated for the reduction of elevated intraocular pressure in patients with open angle glaucoma or ocular hypertension.
FULL PRESCRIBING INFORMATION **Indications and Usage** 1. RHOPRESSA™ (netarsudil ophthalmic solution) 0.02% is a Rho kinase (b) (4) inhibitor indicated for the reduction of elevated intraocular pressure in patients with open-angle glaucoma or ocular hypertension.	**FULL PRESCRIBING INFORMATION** **Indications and Usage** 1. RHOPRESSA™ (netarsudil ophthalmic solution) 0.02% is a Rho-*associated protein* kinase (b) (4) -inhibitor indicated for the reduction of elevated intraocular pressure in patients with open-angle glaucoma or ocular hypertension.
8. Use in Specific Populations **8.1 Pregnancy** Risk Summary (b) (4)	**8. Use in Specific Populations** **8.1 Pregnancy** Risk Summary There are no available data on RHOPRESSA™ use in pregnant women to inform any drug associated risk; however, systemic exposure to netarsudil from ocular administration is low [*see Clinical Pharmacology (12.3)*]. Intravenous administration of netarsudil to pregnant rats and rabbits during organogenesis did not produce adverse embryofetal effects at clinically relevant systemic exposures [see Data]. Data *Animal Data* Netarsudil administered daily by intravenous injection to rats during organogenesis caused abortions and embryofetal lethality at doses ≥0.3 mg/kg/day (126-fold the plasma exposure at the recommended ophthalmic dose [RHOD], based on C_{max}). The NOAEL for embryofetal development

[1] The exposure of AR13324 at RHOD was at or below the LLOQ (0.100 ng/mL). The LLOQ was used to derive exposure margins in nonclinical sections of the labeling.

8.2 Lactation Risk Summary (b) (4)	toxicity was 0.1 mg/kg/day (40-fold the plasma exposure at the RHOD, based on C_{max}). Netarsudil administered daily by intravenous injection to rabbits during organogenesis caused embryofetal lethality and decreased fetal weight at 5 mg/kg/day (1480-fold the plasma exposure at the RHOD, based on C_{max}), Malformations were observed at ≥3 mg/kg/day (1330-fold the plasma exposure at the RHOD, based on C_{max}), including thoracogastroschisis, umbilical hernia and absent intermediate lung lobe. The NOAEL for embryofetal development toxicity was 0.5 mg/kg/day (214-fold the plasma exposure at the RHOD, based on C_{max}). **8.2 Lactation** Risk Summary There are no data on the presence of RHOPRESSA™ in human milk, the effects on the breastfed infant, or the effects on milk production. However, systemic exposure to netarsudil following topical ocular administration is low [*see Clinical Pharmacology (12.3)*], and it is not known whether measurable levels of netarsudil would be present in maternal milk following topical ocular administration. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for RHOPRESSA™ and any potential adverse effects on the breast-fed child from RHOPRESSA™.
12. Clinical Pharmacology **12.1 Mechanism of Action** (b) (4)	**12. Clinical Pharmacology** **12.1 Mechanism of Action** (b) (4)
11. Description Netarsudil is a Rho kinase (b) (4) inhibitor…	**12. Description** Netarsudil is a Rho-*associated protein* kinase (b) (4) inhibitor…
13. Nonclinical Toxicology	**13. Nonclinical Toxicology**

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility	13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
Long-term studies in animals have not been performed to evaluate the carcinogenic potential of netarsudil. Netarsudil was not mutagenic in the Ames test, in the mouse lymphoma test, or in the *in vivo* rat micronucleus test. Studies to evaluate the effects of netarsudil on male or female fertility in animals have not been performed.	No edits are recommended.

2 Drug Information

2.1 Drug

CAS Registry Number: 1422144-42-0

Generic Name: Netarsudil mesylate

Code Name: AR-13324

Chemical Name:

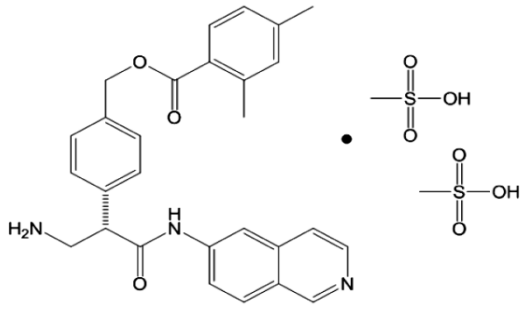
- Benzoic acid, 2,4-dimethyl-, [4-[(1*S*)-1-(aminomethyl)-2-(6-isoquinolinylamino)-2-oxoethyl]phenyl]methyl ester, methanesulfonate (1:2)
- (*S*)-4-(3-amino-1-(isoquinolin-6-yl-amino)-1-oxopropan-2-yl)benzyl 2,4 dimethylbenzoate dimesylate

Molecular Formula/Molecular Weight

Chemical form	Molecular Formula	Molecular Weight
Dimesylate salt Free base	$C_{30}H_{35}N_3O_9S_2$ $C_{28}H_{27}N_3O_3$	645.74 g/mol 453.53 g/mol

Structure: See Figure 1.

Figure 1: Chemical Structure of Netarsudil (AR-13324)



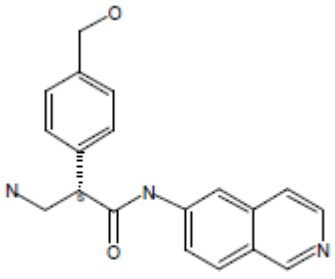
Description of Netarsudil (AR-13324)-Related Compounds:

- Netarsudil is composed of a single enantiomer (S-enantiomer). The chirality arises in the *alpha* carbon of the *beta* amino acid.

(b) (4)

- Metabolism of netarsudil by esterases produces the metabolite AR-13503 (Figure 2).

Figure 2: Chemical Structure of AR-13503 (Main Netarsudil Metabolite)



AR-13503 (*S*)

- (b) (4)

- (b) (4)

Pharmacologic Class: Rho-associated protein kinase inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

The following IND and DMFs were listed in form 356h:

IND 113064

(b) (4) DMF (b) (4)
(b) (4) DMF (b) (4)
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(b) (4) DMF (b) (4)

2.3 Drug Formulation

The composition of the drug product on a per milliliter (mL) basis is shown in Table 1. The netarsudil mesylate content is specified as the free base of the drug substance.

Table 1: Composition and Components of Netarsudil Ophthalmic Solution 0.02%

Component	Function	Netarsudil Concentration: 0.2 mg/mL	
		Quantity per mL (mg)	Quantity (% w/v)
Netarsudil mesylate	Active Ingredient	0.2 (b) (4)	0.02
Mannitol	(b) (4)		
Boric acid			
Benzalkonium chloride	Preservative	(b) (4)	0.015
Sodium hydroxide[3]	pH Adjuster	as needed	as needed
Water for Injection	(b) (4)	q.s.	q.s.
Total		**1 mL**	**100%**

(b) (4)

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

The applicant test article formulations used in the long-term ocular toxicity studies were spiked with 5% (b) (4) and 0.5% of each (b) (4) Therefore, the (b) (4) and these 3 impurities are considered qualified up to 5% and 0.5% administered 2x/day, respectively. See further details under Section 6.2 Repeat Dose Toxicity of this review.

2.6 Proposed Clinical Population and Dosing Regimen

RHOPRESSA™ is indicated for the reduction of elevated intraocular pressure in patients with open-angle glaucoma or ocular hypertension. The intended dose regimen is one drop in the affected eye(s) once daily in the evening.

2.7 Regulatory Background

This NDA is a resubmission after withdrawal. No new nonclinical information was submitted. The nonclinical information reviewed herein was submitted in the original NDA. Some milestones during the drug development process pertinent to the nonclinical discipline are listed below:

- Type B EOP2 CMC meeting held on 3-10-14 (SD # 22 IND 113064) – The Division agreed that no new nonclinical studies were required to qualify the impurities of the test article produced by (b) (4) which was proposed to be used in Phase 3 studies. The Division provided recommendations to the applicant regarding qualification of genotoxic impurities (b) (4)
- Type B EOP2 meeting held on 4-11-14 (SD# 23 IND 113064) - The Division agreed that the battery of nonclinical studies appeared sufficient for NDA submission. The same recommendations given on 3-10-14 were conveyed to the applicant regarding qualification of genotoxic impurities. The Division informed the applicant that they needed to submit a waiver to justify the omission of carcinogenicity studies.
- Type B Pre-NDA meeting held on 10-27-15 (SD # 80 IND 113064) – The applicant sought Division advice on an in vitro assay to explore whether AR-13324 induces corneal phospholipidosis. The Division agreed to the approach.
- Type B Pre-NDA CMC meeting (SD # 88) – CMC and nonclinical review teams agreed with the applicant on proposed specifications for drug substance and drug product impurities. Based on the topical ocular route of administration (potentially resulting in lower ocular exposures), single daily dose administration, and low levels expected at specifications consistent with ICH Q3A and/or Q3B guidances, additional assessments of potential mutagenicity for several impurities (Tables 11 and 12 of SD # 88 briefing document) was not anticipated. The Division agreed

with the justification for the proposed specification of NMT [redacted] for the sum of [redacted] in the drug substance.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacology

- Inhibition of Human Protein Kinase Catalytic Activities by AR-13324, [redacted] AR-13503, [redacted] and AR-12286 (Study # AR-13324-IPH01)
- Effect of 0.04% AR-13324 Solution on Aqueous Humor Dynamics in Normal Monkey Eyes (Study # AR-13324-APH07)
- A 10-Day Study to Compare the Efficacy and Tolerability of [redacted] and AR-[redacted] Following Topical Ocular Administration in Dutch Belted Rabbits (Study # AR-13324-APH01)
- A 10-Day Study to Compare the Efficacy and Tolerability of 0.02% and 0.04% solutions of AR-13324 in Formulation CF324-01 Following Topical Ocular Administration in Dutch Belted Rabbits (Study # AR-13324-APH04)
- Dose Response Evaluation of AR-13324 in Clinical Formulation CF324-01 Following Topical Ocular Administration in Dutch Belted Rabbits (Study # AR-13324-APH05,
- A Crossover Study to Compare the Efficacy and Tolerability of Solutions of 0.08% [redacted] and 0.04% AR-13324 Following Topical Ocular Administration in Normotensive Formosan Rock Monkeys (Study # AR-13324-APH02)
- Dose Response Evaluation of AR-13324 in Clinical Formulation CF324-01 Following Topical Ocular Administration in Normotensive Formosan Rock Monkeys (Study # AR-13324-APH06
- Effect of AR-13324 and Related Compounds on Cytoskeletal Components of Trabecular Meshwork Cells in Vitro (Study # AR-13324-IPH04)
- Evaluation of the Effect of AR-13324M on Aqueous Flow Pattern and Morphology in Enucleated Eyes (Study # AR-13324-IPH05)
- Effect of AR-13324 on Episcleral Venous Pressure (EVP) in Dutch-Belted Rabbits: Part 1 and Part 2 (Study # AR-13324-APH08)

Secondary Pharmacology

- Human Protein Kinase Inhibitory Profiles of [redacted] AR-13324, [redacted] (Study # AR-13324-IPH02)
- Non-Kinase Selectivity Profiling of the Rho-kinase Inhibitors [redacted], [redacted] and AR-13324 (Study # AR-13324-IPH03)

Safety Pharmacology

- hERG Potassium Channel Inhibition Data from Study # AR-13324-IPH03: Non-Kinase Selectivity Profiling of the Rho-kinase Inhibitors [redacted] and AR-13324
- Effect of AR-13324 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (Study # AR-13324-IS03)

- Evaluation of Cardiovascular (Hemodynamic) Function in Conscious Telemetered Beagles Following Intravenous Administration of AR-13324 (Study # AR-13324-AS08)

PK/ADME

- Liver S9 Extracts from Rat and Human; Liver Microsomes from Various Species: Porcine Cornea (Study # AR-13324-IPK03)
- In vitro Binding of AE-13324 and AR-13503 to Human, Beagle, and Rat Plasma (Study of AR-13324-IPK01)
- Binding of AR-13324 and AR-13503 to Melanin In Vitro (Study # AR-13324-IPK02)
- Binding of AR-13324 and AR-13503 to Melanin *In Vitro* (Study # AR-13324-IPK02)
- Ocular Tissue Distribution Study of Total Radioactivity in Dutch-Belted Male Rabbits Following a Single Topical Ocular Administration of [^{14}C]AR-13324 (Study # AR-13324-APK03)
- Excretion Mass Balance and Quantitative Whole-Body Autoradiography in Long-Evans Rats Following a Single Intravenous Dose of [^{14}C]AR-13324 (Study AR-13324-APK04)
- Determination of AR-13324 and AR-13503 in Aqueous Humor following Topical Ocular Administration of 0.02% AR-13324 in New Zealand Rabbits (Study # AR-13324-APK05)

Single-Dose Toxicity

- Acute Intravenous Toxicity Study in Rats with AR-13324 (Study # AR-13324-AS01)
- A Non-GLP Escalating Dose Acute Intravenous Toxicity Study in Dogs with AR-13324 (Study # AR-13324-AS02)

Repeat-Dose Toxicity

Ocular Route:

- A 7-Day Ocular Toxicity Study of AR-13324 Ophthalmic Solutions in Dutch Belted Rabbits with a 7-Day Recovery (Study # AR-13324-AS05)
- A 7-Day Ocular Toxicity Study of AR-13324 in Cynomolgus Monkeys with a 7-Day Recovery Period (Study # AR-13324-AS06)
- A 7-Day Ocular Toxicity Study of AR-13324 in Cynomolgus Monkeys (Study # AR-13324-AS07)
- A 28-Day GLP Ocular Toxicity Study of AR-13324 Ophthalmic Solution in Dutch-Belted Rabbits with a 14-Day Recovery Period (Study # AR-13324-AS11)
- 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 (Study # AR-13324-AS14)
- A 3/6-Month Ocular Toxicity Study with AR-13324 Ophthalmic Solutions in Dutch Belted Rabbits (Study # AR-13324-AS15)
- A 1-Month Ocular Toxicity Study of AR-13324 in Cynomolgus Monkeys with at 2-Week Recovery Period (Study # AR-13324-AS11)

Systemic Route:

- A 7-Day GLP Intravenous Toxicity Study of AR-13324 in Rats with a 7-Day Recovery (Study # AR-13324-AS03)
- A 7-Day GLP Intravenous Toxicity Study of AR-13324 in beagle Dogs (Study # AR-13324-AS04)
- A 28-Day GLP Intravenous Toxicity Study of AR-13324 in Rats with a 14-Day Recovery (Study # AR-13324-AS09)
- A 28-Day GLP Intravenous Toxicity Study of AR-13324 in Beagle Dogs with a 14-Day Recovery (Study # AR-13324-AS10, GLP, Module 4.2.3.2)

Genetic Toxicology

- Bacterial Reverse Mutation Assay (Study # AR-13324-IS01)
- In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK+/- Mouse Lymphoma Assay (Study # AR-13324-IS02)
- Micronucleus Test Performed as Part of Study AR-13324-AS03: A 7-Day GLP Intravenous Toxicity Study of AR-13324 in Rats with a 7-Day Recovery (Study # AR13324-IS03)

Reproductive and Developmental Toxicology

- Study for Effects of AR-13324 on Embryo-Fetal Development in Rats (SEG II) Following Intravenous Administration via a Vascular Access Port (VAP) (Study # AR-13324-AS21)
- A Dose-Range-Finding Study for Effects of AR-13324 Following Intravenous Administration via an Indwelling Jugular Vein Catheter on Embryo-Fetal Development in Rats (SEG II) (Study # AR-13324-AS18)
- Study for Effects of AR-13324 on Embryo-Fetal Development in Rabbits (SEG II) Following Intravenous Administration via a Vascular Access Port (VAP) (Study # AR-13324-AS22)
- A Dose-Range-Finding Study for Effects of AR-13324 Following Intravenous Administration via a Vascular Access Port on Embryo-Fetal Development in Rabbits (SEG II) (Study # AR-13324-AS20)

Special Toxicology Studies

- Evaluation of the Potential of AR-13324 to Induce Phospholipidosis in a Cell-Based Assay (Study # AR-13324-IPH07)

3.2 Studies Not Reviewed

- Comparative Efficacy and Tolerability of Solutions of 0.04% AR-13324 in Four Formulations Varying (b) (4)
  Topical Ocular Administration in Dutch Belted Rabbits (Study # AR-13324-APH03)
- Pharmacokinetics Data from Liquid Chromatography/Mass Spectrometry/Mass Spectrometry Analysis of Rat Plasma Samples Following Acute Intravenous Administration of AR-13324 (Study # AR-13324-APK01)

- Pharmacokinetic Data from the Rising Dose Tolerance Study of AR-13324 Administered by Intravenous Infusion to Dogs (Study # AR-13324-APK02)
- All analytical methods and validation reports (Module 4.2.2.1)

3.3 Previous Reviews Referenced

IND 113064 nonclinical reviews:

- Initial IND review – Filed in DARRTS on 3-7-2012
- Review SD # 9, 16, 25, and 26 (several toxicology studies including the 28-day ocular and IV studies/PK/ADME studies)- Filed in DARRTS on 5-23-2014
- Review SD # 59 (Ocular tissue distribution and 3-month rabbit studies with fixed dose combination) - filed in DARRTS on 9-2-2015
- Review SD # 80 (Assay for phospholipidosis)- Filed in DARRTS on 10-20-2015

4 Pharmacology

4.1 Primary Pharmacology

Most studies were reviewed under the initial review of IND 113064. The main findings include:

- AR-13324 (netarsudil) has potent inhibitory activity against ROCK1 and ROCK2 (K_i =1.1 nM and 1.2 nM, respectively). Assays were designed to measure inhibition of the phosphoryltransferase activity of the kinase.
    - The K_i is equivalent to 0.498 ng/mL, based on free base.
- Its primary metabolite, the ester hydrolysis product AR-13503, is an active metabolite with at least 5-fold greater *in vitro* activity against both Rho kinases than AR-13324 (K_i = 0.2 nM for both ROCK1 and ROCK2).
    - The K_i is equivalent to 0.0612 ng/mL, based on an estimated molecular weight of 306 g/mol.
- In normotensive female cynomolgus monkeys treated with a single drop of 0.04% AR-13324 (clinical formulation) into one eye, AR-13324 appeared to have a dual mechanism of IOP reduction; increase in tonographic outflow facility and decrease of aqueous humor flow rates (i.e., decreased rate of aqueous humor secretion by the ciliary body). IOP and outflow facility were measured at 6 hours postdose, and aqueous humor flow rates was measured hourly for 6 hours postdose.
    - Mean IOP was decreased by ~25% ($p<0.005$) when compared with contralateral vehicle-treated eyes or baseline values.
    - Mean outflow facility was increased ($p<0.05$) by 53% in drug-treated eyes compared with either contralateral vehicle-treated eyes or baseline measurement.
    - Mean aqueous humor flow rate was reduced ($p<0.05$) by 20% and 23% when compared with contralateral vehicle-treated eyes and baseline values, respectively.

Note: *This study supports* [redacted] *in section 12.1 Mechanism of Action of the proposed label: increasing trabecular outflow facility* [redacted].

- IOP reduction was also observed in normotensive Dutch-Belted rabbits administered a single drop of AR-13324 0.02% and 0.04% solutions for 10 days (not the clinical formulation).
  - The maximal IOP reductions observed for 0.02% and 0.04% of AR-13324 were 7.0 mmHg and 7.1 mmHg, respectively at 4 hours postdose; and 1.3 mmHg and 1.5 mmHg, respectively at 24 hours postdose.
  - Both 0.02% and 0.04% solutions of AR-13324 produced trace (+0.1-+0.6) hyperemia that typically lasted 4-8 hours after each dose.
  - The average hyperemia severity scores were higher on Days 1-3 and progressively decreased throughout the study in both treatment groups.
- In Dutch-Belted rabbits, a dose-dependent IOP lowering effect was observed at AR-13324 solution concentrations of 0.005%, 0.01%, 0.02%, and 0.04% (clinical formulation) with peak occurring at 4-6 hours postdose throughout the study. A single drop of the test article was administered once daily for 3 days.
  - The maximum IOP reduction produced by the 0.005%, 0.01%, 0.02%, and 0.04% solutions of AR-13324 were 2.5 ± 0.2 mmHg, 4.6 ± 0.2 mmHg, 5.0 ± 0.6 mmHg, and 8.1 ± 0.7 mmHg, respectively.
  - All solutions produced trace to mild (+0.1 - +0.6) hyperemia that typically lasted 4-8 hours postdose.
- In normotensive Formosan Rock Monkeys, a dose-dependent IOP lowering effect was observed at AR-13324 solution concentrations of 0.01%, 0.02%, and 0.04% (clinical formulation). A single drop of the test article was administered once daily for 3 days.
  - The maximum IOP reduction produced by the 0.01%, 0.02%, and 0.04% solutions of AR-13324 were 4.2 ± 0.2 mmHg, 5.8 ± 0.3 mmHg, and 7.5 ± 1.1 mmHg, respectively.
  - None of the treatments caused ocular irritation.

Additional Primary Pharmacology studies not previously reviewed include:

Effect of AR-13324 and Related Compounds on Cytoskeletal Components of Trabecular Meshwork Cells in Vitro (Study # AR-13324-IPH04) – The parent compound AR-13324 and the metabolite AR-13503 demonstrated the greatest potency in disrupting actin stress fibers in porcine trabecular meshwork (PTM) cells and focal adhesions inhuman trabecular meshwork (HTM) cells. These findings are shown in Table 2.

Table 2: IC_{50} Values for AR-13324 and Related Compounds in PTM and HTM Cell-Based Assays

Compound ID	PTM IC_{50} (nM)	HTM IC_{50} (nM)
AR-13324	504	219
(b) (4)	7586	2247
	1727	246
AR-13503	102	64

Note: A concentration of 219 nM AR-13324 is equivalent to 99.3 ng/mL free base.

AR-13503, the esterase metabolite of AR-13324, had approximately 4- to 5-fold greater activity than AR-13324 in both cell-based assays. (b) (4) was ~15-fold less active in PTM cells and ~10-fold less active in HTM cells compared to AR-13324. (b) (4) was not as effective as the single enantiomer AR-13324 in the PTM cell-based assay.

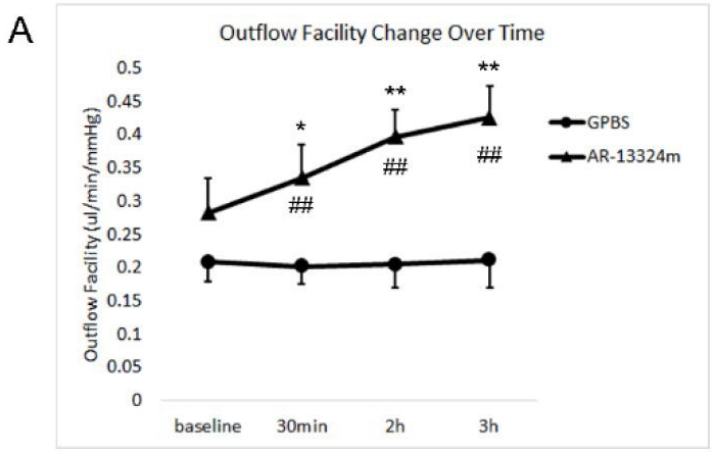
As noted by the applicant, these results support the hypothesis that inhibition of Rho kinase in the anterior chamber of the eye causes relaxation of the trabecular meshwork, the tissue responsible for regulating aqueous humor outflow. Relaxation of the trabecular meshwork should then decrease resistance to conventional outflow and lower intraocular pressure.

Evaluation of the Effect of AR-13324M on Aqueous Flow Pattern and Morphology in Enucleated Eyes (Study # AR-13324-IPH05) – The study investigated the effects of AR-13503 (referred in this report as AR-13324M), the active metabolite of AR-13324, on the outflow facility, hydrodynamics, and morphology of the trabecular outflow pathway in enucleated human eyes. To label the hydrodynamic outflow, anterior chamber fluid from all eyes was exchanged for 5 mL of perfusate (GPBS) containing fluorescent microspheres followed by perfusion of fixed volume (150 µL) of the same solution.

AR-13503 increased outflow facility by increasing the area of actively filtering tissue in the trabecular outflow pathway, expanding the trabecular meshwork (TM), and dilating the episcleral veins (ESVs) in normal human eyes, as noted in more detail below.

- The outflow facility of AR-13503-treated eyes was significantly higher compared to GPBS control after 30 min perfusion (Figure 3).

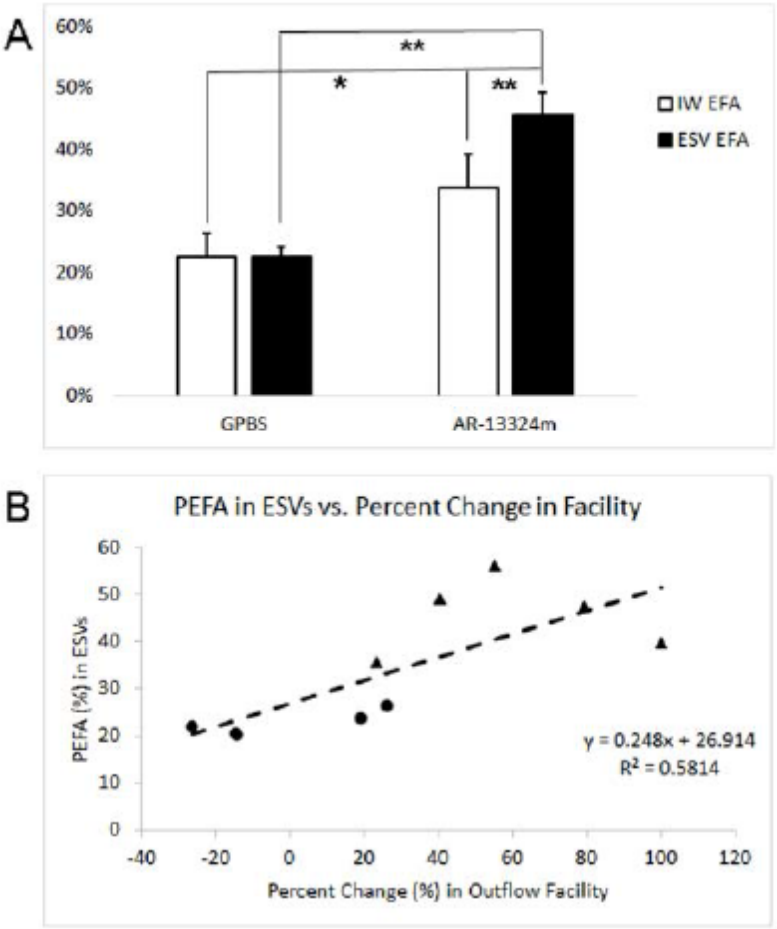
Figure 3: AR-13503 (AR-13324M) Outflow Facility over Time in Human Enucleated Eyes



AR-13503 treatment significantly increased outflow facility over time (one tailed, paired t-test). Treated eyes showed significantly increased outflow facility after 30 min perfusion when compared to the controls (##: $p<0.01$). There was also a significant increase when compared to its own baseline (*: $p<0.05$; **: $p<0.01$).

- Effective filtration area (EFA) increased significantly in ESVs and at inner wall (IW) of Schlemm's canal (Figure 4A). The percent of the scleral or TM surface that showed fluorescent tracers was termed as the effective filtration area (EFA).
    - There was a significant positive correlation between episcleral veins effective filtration area and % change in outflow facility (Figure 4B).

Figure 4: Effects of AR-13503 (AR-13324M) on Episcleral Veins and Schlemm's Canal Internal Wall Effective Filtration Area



AR-13324M treatment induced a significant increase in both ESV and IW EFA when compared to controls respectively **(A)**. While ESV EFA and IW EFA remained similar to each other in control eyes, ESV EFA in treated eyes showed a significant increase when compared to its IW EFA (*: $p<0.05$; **: $p<0.01$) ($n=5$). Furthermore, ESV EFA was found to have a positive correlation with percent change in outflow facility **(B)** (*R square*= 0.58; p =0.01; solid circle: control eyes; solid triangular shape: AR- 13324M treated eyes).

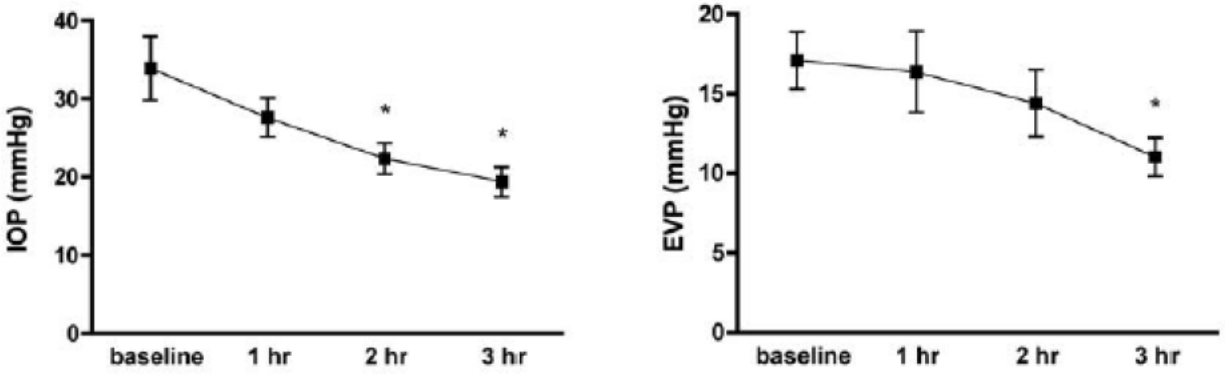
- AR-13503 treatment significantly increased the average size of ESVs when compared to controls (3735 ± 405 vs. 2231 ± 217 square µm; p=0.001).

- Trabecular meshwork results revealed an expansion of the trabecular meshwork in low tracer regions in treated eyes.

Effect of AR-13324 on Episcleral Venous Pressure (EVP) in Dutch-Belted Rabbits: Part 1 and Part 2 (Study # AR-13324-APH08) – In Part 1 of this study, 6 male Dutch Belted rabbits were dosed with Netarsudil Ophthalmic Solution 0.04% QD (AM) for 3

days. On Day 3, recordings were taken predose and for 3 hours after dosing for IOP, EVP, arterial pressure, heart rate, and carotid blood flow. IOP was reduced by 39% ± 7% in the netarsudil-treated rabbits compared to baseline at 3 hours postdose. EVP decreased by 35% ± 4% in the netarsudil-treated rabbits. Arterial pressure, heart rate, and carotid blood flow were unchanged following treatment with netarsudil.

Figure 5: Effects of Topical Ocular AR-13324 on Intraocular Pressure and Episcleral Venous Pressure in Dutch-Belted Rabbits



Values are mean ± SE; *significant difference from baseline

In Part 2 of the study, 5 male rabbits were dosed with vehicle. IOP and EVP were reduced by 24% ± 4% and increased by 25% ± 5%, respectively at 3 hours postdose, compared to baseline. When compared to time-matched vehicle controls, the decrease in IOP and EVP in netarsudil-treated rabbits was 21% and 42%, respectively, at 3 hours.

Note: *Studies # AR-13324-IPH05 and # AR-13324-APH08 support the* (b) (4)

4.2 Secondary Pharmacology

Per initial IND review, netarsudil mesylate and related compounds (b) (4) have demonstrated inhibitory activity against a number of additional human kinases *in vitro,* besides ROCK1 and ROCK2.

Human Protein Kinase Inhibitory Profiles of (b) (4)**, AR-13324,** (b) (4) **(Study # AR-13324-IPH02; GLP; Module 4.2.1.2):** When screened at a concentration of 0.5 μM (227 ng/mL), AR-13324 inhibited activity of 25 additional kinases in a competitive binding assay. Inhibition of protein kinase activity was considered significant if the test article reduced probe binding by >65%.

Non-Kinase Selectivity Profiling of the Rho-kinase Inhibitors (b) (4) **and AR-13324 (Study # AR-13324-IPH03; non-GLP; Module 4.2.1.2):** When screened at a concentration of 10 μM (4535 ng/mL), AR-13324 (netarsudil) showed significant binding inhibitory activity against more than 20 non-kinase proteins including the human norepinephrine transporter (hNET), the human serotonin transporter (hSERT), dopamine receptor, adenosine receptor, alpha 2A adrenergic receptor, histamine H1 and H2 receptors, cannabinoid CB1 receptor, opiate kappa and mu receptors, L-type calcium channels, hERG potassium channel, and all five cytochrome P450 isoforms tested (2C19, 2D6, 3A4, 1A2, 2C9). Significant inhibitory activity was defined as 50% or greater inhibition of binding or enzymatic activity in the assay. All assays were competitive binding assays, except the CYP450 assays which were enzyme inhibition assays.

In a functional assay in HEK-293 cells recombinantly over-expressing the hNET, AR-13324 did not show significant inhibitory activity (IC_{50} > 20 μM) of hNET reuptake of a fluorophore-labeled biogenic amine, under the conditions of the cell-based assay.

In in the study report, it was concluded that AR-13324 can (b) (4)

Note: *In the Pharmacology Written Summary, the applicant states the following:* (b) (4)

However, the following observations (b) (4) *is a relevant pharmacological effect for the proposed indication:*

- (b) (4)

Therefore, this reviewer believes that based on these observations, (b) (4) [redacted]

The applicant claims the clinical exposure of AR-13324 was below the lower limit of quantitation of 0.100 ng/mL, except for one subject with levels of 0.11 ng/mL for the primary metabolite (Section 12.2 of the proposed label). Therefore, it is unlikely that inhibitory activity against secondary kinase targets or non-kinase targets would have any detectable systemic pharmacological effect at the proposed ocular dosing regimen in humans.

4.3 Safety Pharmacology

Draft reports of these studies were reviewed under the initial review of IND 113064. There were no significant differences in the data between draft and final study reports.

Central Nervous System: There was no effect in rats at doses of 1, 3, or 10 mg/kg IV measured by a functional observation battery (FOB) during the 7-day repeated dose GLP study (Study # AR-13324-AS03). The FOB was performed prior to treatment on Day 1 and 30 minutes post treatment on Day 7. There were no effects in body temperature with acute doses in telemetered dogs at 1, 12.5, or 25 mg/kg IV (Study # AR-13324-AS08) or in rats during repeated doses over 7 days (Study # # AR-13324-AS03).

Cardiovascular:

Effect of AR-13324 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (Study # AR-13324-IS03; GLP; Module 4.2.1.3) - AR-13324 statistically significantly inhibited hERG current by 43.6 ± 2.7% (mean ± SD) at 0.3 µM, 63.0 ± 6.8% at 0.8 µM, and 92.9 ± 2.9% at 2.9 µM, in comparison to 0.9 ± 2.3% in the control group. The IC_{50} was 0.4 µM (181 ng/mL using a MW of the free base of 453.54 g/mol). The positive control (60 nM terfenadine) inhibited hERG current by 79.9 ± 2.4%.

Note: *The batch of AR-13324 used in this study (# 431190) contained an intentionally high level* (b) (4) [redacted] *per Certificate of Analysis.*

The concentrations tested in this study, 0.3, 0.8, and 2.9 µM, are equivalent to 136, 363, and 1315 ng/mL, respectively, using a molecular weight of the free base of 453.54 g/mol for AR-13324.

Evaluation of Cardiovascular (Hemodynamic) Function in Conscious Telemetered Beagles Following Intravenous Administration of AR-13324 (Study # AR-13324-AS08; GLP; Module 4.2.1.3) - Male Beagle dogs (3/treatment), implanted with telemetry transducers, were administered two doses (0 and 1 mg/kg; 12.5 and 25 mg/kg; free based equivalent of 0, 0.7, 8.8, and 17.6 mg/kg, respectively) by a 15 min IV infusion. There was a washout period of at least 7 days between the 2 doses. Changes in heart rate, arterial

pressure (diastolic, systolic and mean), and ECG were evaluated up to 24 hours postdose. Body temperature and drug plasma levels (collected at 30 minutes postdose) were also measured.

One animal at 12.5 mg/kg had a swollen right leg at 24 hours post-dose. At 25 mg/kg, decreased activity, food particle emesis (2/3 animals), squinting of the eyes, and redness of the legs, ears, abdomen and sclera were observed in all 3 animals starting during dosing through 24 hours postdose. Loose/soft feces were also observed in all three animals at 24 hours postdose.

Doses of 12.5 and 25 mg/kg were associated with increased heart rate through 20 hours postdose (maximum increase of 57% and 124%, respectively, compared to corresponding vehicle value). Decreased arterial pressure (diastolic, systolic and mean) was observed through 5 hours postdose at 12.5 mg/kg. At 25 mg/kg, decreased arterial pressure was observed throughout the 24-hour duration of the measurement period. The maximum decrease in mean arterial pressure at 12.5mg/kg and 25 mg/kg was 23% and 36%, respectively, from corresponding vehicle value.

AR-13324 had no effects on QTc at doses up to 25 mg/kg.

Redness in various body parts and decreased blood pressure are expected pharmacological actions of Rho-kinase inhibitors. Rho kinase inhibitors reduce blood pressure by decreasing vascular wall smooth muscle contractility and thereby reducing vascular tone, causing vasodilation. The redness is a sign of peripheral vasodilation.

Toxicokinetics data indicated that all animals dosed with AR-13324 had significant exposure to AR-13324 and measurable exposure to [redacted] (b) (4) an intentional impurity in this lot of AR-13324. AR-13503 (AR-13324 primary metabolite) and [redacted] (b) (4) were also detected but at considerably lower levels. The average concentrations at 30 minutes postdose (only timepoint evaluated) are shown below:

Table 3: Average Plasma Concentrations of AR-13324 and Related Compounds in Dog Cardiovascular Safety Pharmacology Evaluation

Dose (**mg/kg free base**)	Average Concentration at 30 minutes (ng/mL)				Ratio (b) (4) / 13324	Ratio (b) (4) / 13503
	AR-13324	(b) (4)	AR-13503	(b) (4)		
0.7	26.6	3	BLQ	BLQ	0.113	CND
8.8	412.3	49.9	11.8	7.91	0.121	0.670
17.6	871.7	108.2	42.8	24.8	0.124	0.579

BLQ = below level of quantitation, CND = could not be determined

Note: *The batch of AR-13324 used in this study (# 0431190) contained an intentionally high level* [redacted] (b) (4) *per Certificate of Analysis.*

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The main findings of studies reviewed under the initial review of IND 113064 include the following:

Liver S9 Extracts from Rat and Human; Liver Microsomes from Various Species: Porcine Cornea (Study # AR-13324-IPK03; Module 4.2.2.4) - The extent of ester cleavage differed between species and followed the rank order: male rabbits (91%) > male and female mice (72 and 85%, respectively) > male and female monkeys (46 and 54%, respectively) > pooled human microsomes (45%) > male and female rats (28 and 4%, respectively), > male and female beagle dogs (-4.6 and 0.8%, respectively).

Microsomes from all testes species showed little ability to metabolize AR-13503.

Corneas from different species showed rapid metabolism of AR-13324 in dogs (23% remaining) followed by rabbits (30% remaining), pigs (36% remaining), monkeys (48% remaining), and humans (60% remaining) after a 4-hour incubation. When normalized to a 7-mm circular section of cornea, the $t_{1/2}$ were 98 min (dog), 109 min (monkey), 140 min (rabbit), 156 min (pig) and 175 min (human).

Plasma from different species showed little metabolism of AR-13324 in human, dog, monkey, and rat in after a 3-hour incubation (>88% of AR-13324 remaining). In contrast, rapid metabolism was observed in rabbit plasma (17% remaining).

***In vitro* Binding of AE-13324 and AR-13503 to Human, Beagle, and Rat Plasma (Study # AR-13324-IPK01, Module 4.2.2.3)** – AR-13324 showed high protein binding (97-100% at concentrations of 10, 100, and 1000 μM). Less protein binding was observed with metabolite AR-13503. Binding percentage for 10, 100, and 1000 μM were 60-70% for human plasma, 43-45% for beagle plasma, and 47-50% for rat plasma.

Binding of AR-13324 and AR-13503 to Melanin *In Vitro* (Study # AR-13324-IPK02, Module 4.2.2.3) – AR-13324 and AR-13503 (active metabolite) showed similar affinities for cuttlefish melanin (K_d = 23 μM and 20 μM, respectively). The positive control, chloroquine, showed a K_d of 14 μM. Ciprofloxacin (published to have low affinity for melanin) showed a K_d of 35 μM.

Ocular Tissue Distribution Study of Total Radioactivity in Dutch-Belted Male Rabbits Following a Single Topical Ocular Administration of [^{14}C]AR-13324 (Study # AR-13324-APK03; Module 4.2.2.3) - Rabbits were given a single topical ocular dose of 0.02% [^{14}C]AR-13324 at a dose volume of 0.035 mL per eye (both eyes). The rank order (based on C_{max}) of ocular tissue radioactivity concentrations was as follows: cornea (3666 ng•eq/g) > conjunctiva (2442 ng•eq/g) >> iris/ciliary body (955 ng•eq/g) >> retina-choroid-plexus (79.8 ng•eq/g) > aqueous humor (16.3 ng•eq/g) > vitreous humor (7.27 ng•eq/g) > lens (1.77 ng•eq/g). The highest blood (0.70 ng•eq/g) and plasma (0.88 ng•eq/g) concentrations were found at 0.25-0.5 hours after dosing.

The T_{max} occurred between 0.25 to 1 hours in most ocular tissues, except for a T_{max} of 8 hours in the aqueous humor (both eyes) and iris/ciliary body (0.25 hours in the left eye; 8 hours in the right eye). The $t_{1/2,e}$ values ranged from 12 to 27 hours for most ocular tissues, blood, plasma, liver, and kidney. Terminal elimination from retina-choroid-plexus, lens and iris/ciliary body was much slower with $t_{1/2,e}$ values ranging from 68 to 204 hours. The prolonged $t_{1/2}$ in uveal tissues may be related to an association with melanin.

Note: *The notable difference in T_{max} in the right and left eye iris/ciliary body is due to significant within group variability at the 0.25-hour and 8-hour timepoint. At each timepoint, one (out of 3) eyes showed a value significantly higher (37X for the left eye; 17X for the right eye) than the mean of the other 2 eyes within the group.*

Approximately 60 ± 19.0% of the dosed radioactivity was recovered in the excreta by 24 hours (~48 ± 17.5% in the feces and ~12 ± 1.5% in the urine). By 48 hours post-dose, approximately 80 ± 13.3% of the dosed radioactivity was recovered in the excreta (86-88% in two rabbits and 64% in the third rabbit). The % of dose remaining in all ocular tissues after 48 hours was 0.68 ± 0.05%.

Excretion Mass Balance and Quantitative Whole-Body Autoradiography (QWBA) in Long-Evans Rats Following a Single Intravenous Dose of [^{14}C]AR-13324 (Study AR-13324-APK04; Module 4.2.2.3) - Male Long Evans (3 for mass balance; 1 per timepoint up to 168 hours postdose for QWBA) rats were administered a single IV bolus dose of [^{14}C]AR-13324 into the tail vein at a target dose of 1 mg/kg (100 µCi/kg). The primary route of elimination in intact rats was the feces, which accounted for an average of 75.9 % of the administered dose. An average of 14.4 % of the administered dose was recovered in the urine. The total mean recovery of radioactivity by 168 hours postdose averaged 91.0 % of the dose (note: carcasses were not analyzed).

Blood to plasma concentration ratios ranged from 0.90 to 1.75.

The C_{max} of [^{14}C]AR-13324-derived radioactivity in most tissues (26 of 51 tissues) was found at 0.5 hours post-dose. The highest overall concentrations were observed in the lung (high focal concentration; 137.813 µg equiv/g at 96 hours), adrenal gland cortex (14.868 µg equiv/g at 4 hours), liver (high focal concentration; 9.810 µg equiv/g at 0.5 hours), contents of the alimentary canal (27.944 µg equiv/g in cecum at 8 hours, and 33.604 µg equiv/g in small intestine at 4 hours), bile (21.322 µg equiv/g at 0.5 hours), and urine (27.933 µg equiv/g at 0.5 hours), which reflected the routes of elimination for [^{14}C]AR-13324-derived radioactivity.

Most tissues in Long Evans rats had concentrations that were higher than blood and the elimination of radioactivity was not complete at 168 hours, but levels were low (~1 to 20 times LLOQ of 0.011 µg equiv./g tissue), except for adrenal gland cortex, lung, liver, adrenal gland medulla, preputial gland, eye uvea, exorbital lacrimal gland, intra-orbital gland. In some of these tissues (e.g., adrenal gland cortex, preputial gland, uvea) the levels of radioactivity did not decline with time.

Elevated radioactivity concentrations were observed in melanin-containing tissues, such as the eye uvea, pigmented skin, and meninges. The concentration in these tissues did not decline over the study period, which suggested an association of [^{14}C]AR-13324-derived radioactivity with melanin. However, the levels of radioactivity in pigmented and nonpigmented skin were similar at all timepoints, indicating the radioactivity in the skin was not likely related to an association with melanin.

The following additional PK/ADME study was not previously reviewed:

Determination of AR-13324 and AR-13503 in Aqueous Humor following Topical Ocular Administration of 0.02% AR-13324 in New Zealand Rabbits (Study # AR-13324-APK05; Module 4.2.2.4) – Twelve male NZW rabbits were assigned to four treatment groups (n=3/group). In Groups 1 and 2, a single topical ocular dose of 0.02% AR-13324 (Formulation CF324-01; clinical formulation) at a dose volume of 35 µL was administered OU, and subsequently 100 µL samples of aqueous humor were taken at 4 and 6 hours, respectively. In Groups 3 and 4, 0.02% AR-13324 was dosed once daily for three or four days, respectively, and 100 µL samples of aqueous humor were taken 4 hours after the third or the fourth dose (t = 52 hours and t = 76 hours, respectively).

Only one sample from Group 1 had AR-13324 levels (1.82 ng/mL) in the aqueous humor above the LLOQ (0.100 ng/mL). In contrast, AR-13503 (active metabolite) was measurable in all aqueous humor samples. The average concentrations of AR-13503 in aqueous humor from groups 1 – 4 were 7.5 ± 4.87, 3.95 ± 2.53, 6.44 ± 4.15, and 10.74 ± 4.88 ng/mL, respectively. These results indicate that within 4 hours after instillation, AR-13324 was effectively converted to AR-13503 in rabbit aqueous humor.

6 General Toxicology

6.1 Single-Dose Toxicity

These studies were reviewed under the initial review of IND 113064. The following summaries are excerpts from the nonclinical review of IND 113064 review.

Acute Intravenous Toxicity Study in Rats with AR-13324 (Study # AR-13324-AS01; non-GLP; Module 4.3.3.1) - Sprague-Dawley rats (2/sex/group) tolerated AR-13324 dimesylate salt when administered as a single IV dose at 1 or 3 mg/kg in saline. The rats did not tolerate AR-13324 at 45 mg/kg in either saline or mannitol/borate buffer administered 1x/day for 2 days. At this dose, swollen/darkened tails were observed following the first dose (in mannitol/borate buffer) or second dose (in saline). At 45 mg/kg, clinical signs included food crumbling, red staining around the eyes, red staining on the cage paper, decreased activity, hunched posture, ruffed fur coat, scant feces, scant food findings, and pink extremities. The pink extremities may be related to the pharmacology of the test article (vasodilation). All animals at 45 mg/kg in the mannitol/borate buffer were euthanized for humane reasons on Day 2.

A Non-GLP Escalating Dose Acute Intravenous Toxicity Study in Dogs with AR-13324 (Study # AR-13324-AS02; Module 4.3.3.1) – Male and female beagle dogs (1/sex/dose) tolerated AR-13324 dimesylate salt when administered as a single IV infusion over 15 min at 10 or 30 mg/kg. The clinical signs, decreases in body weight and food consumption, and changes in clinical pathology parameters noted at 60 mg/kg AR-13324 were consistent with acute anemia, renal, and hepatic effects. Additional findings at 30 and 60 mg/kg that may have been related to pharmacological effects (vasodilation) of AR-13324 included pink skin and red sclera. Decreased mean blood pressure and increased heart rate were noted at 60 mg/kg. The clinical signs were reversible.

6.2 Repeat-Dose Toxicity

Ocular Route Repeat-Dose Toxicity Studies:

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

Study no.:	AR-13324-AS14
Study report location:	EDR Module 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8-23-2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AR-13324 Ophthalmic Solution 0.01%, lot # BCL545-144B AR-13324 Ophthalmic Solution 0.02%, lot # BCL545-144C AR-13324 Ophthalmic Solution 0.04%, lot # BCL545-144D **Note:** For all three AR-13324 Ophthalmic Solutions, the study report indicates that the purity was assumed to be 100% for dose calculation purposes. Stability data (p. 319, 328, and 337 of the study report) indicates AR-13324 Ophthalmic Solution 0.01%, 0.02%, and 0.04% were 99.1%, 99.7%, and 100.4% label claim at release testing, respectively.

Key Study Findings

- Signs of ocular irritation were observed during the first week of dosing, primarily at the mid and high doses. These included hyperemia, chemosis (less often), and/or discharge (rare).
- One mid-dose and 5 high-dose animals showed reddening of the eye and/or eyelid, as well as partial eye closure. These findings were observed occasionally and resolved with continued dosing.
- Faint or diffuse (i.e., involving the entire cornea) superficial corneal haze was observed in mid-dose and high-dose animals. The corneal haze resolved in all animals before Week 39 despite continued dosing, except for one high-dose female. The corneal haze in this female resolved during the recovery period.
- There was no correlative histologic finding for the corneal haze.
- Slight reductions in mean IOP, the intended pharmacologic activity, were noted at all timepoints and all dose levels. The decrease was reversible.
- Potentially test-article related microscopic findings were limited to increased incidence of lymphoid conjunctival hyperplasia (minimal to mild) at the high dose.
- Plasma levels of the test article and its metabolites were below the limit of quantitation (<1 ng/mL).
- The applicant selected the highest dose as the NOAEL (0.04% BID or 14 µg/dose, BID). Based on the transient nature of the ocular irritation and corneal haze (with no histologic correlates), this reviewer agrees with the applicant's conclusion.
- The NOEL for the finding of corneal haze was the low dose, 0.01% BID.

Methods

Doses:	See Dose Levels table below.
Frequency of dosing:	One drop/eye 2x/day (~8 hours apart ± 5 minutes) for 272 days (39 weeks)
Route of administration:	Topical ocular instillation to both eyes
Dose volume:	~35 µL/drop
Formulation/Vehicle:	AR-13324 Ophthalmic Solution Placebo
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	See Experimental Design table below.
Age:	2.4 to 3.2 years old
Weight:	2.3 to 3.4 kg
Satellite groups:	None
Unique study design:	In Quality section 2.3.P.5 Control of Drug Product, the applicant indicates that the dosing solutions in nonclinical batches used in this study were spiked with (b) (4) at 5%, AR-13324 active metabolite at 0.5%, and impurities (b) (4) at 0.5%. This information was not acknowledged in study report but the Certificates of Analysis confirm the statement.

The placebo solution was not spiked with these impurities, per Certificate of Analysis.

Deviation from study protocol: None with an impact on the validity of the study

Dose Levels:

Group No.	Test Material	AR-13324 Dose per Eye[a]		Approximate Dose Volume per Eye[b]		Dose Conc.[c] per Eye
		(µg/dose)	(µg/day)	(µL/dose)	(µL/day)	(µg/µL)
1	AR-13324 Ophthalmic Solution, Placebo	0	0	35	70	0
2	AR-13324 Ophthalmic Solution, 0.01%	3.5	7	35	70	0.1
3	AR-13324 Ophthalmic Solution, 0.02%	7	14	35	70	0.2
4	AR-13324 Ophthalmic Solution, 0.04%	14	28	35	70	0.4

[a] One drop/eye/dose; 2 doses per day.
[b] Approximate drop volume is 35 µL.
[c] Conc. = concentration of AR-13324 in each ophthalmic solution.

Experimental Design

Group No.	Test Material	No. of Animals					
		Interim Study		Main Study		Recovery Study	
		Males	Females	Males	Females	Males	Females
1	AR-13324 Ophthalmic Solution, Placebo	4	4	4	4	2	2
2	AR-13324 Ophthalmic Solution, 0.01%	3	3	3	3	-	-
3	AR-13324 Ophthalmic Solution, 0.02%	4	4	4	4	2	2
4	AR-13324 Ophthalmic Solution, 0.04%	4	4	4	4	2	2

No. = Number; - = not applicable.
Note: Day 1 was the first day of dosing for all groups. Interim Study animals were dosed through Day 97 and necropsy occurred on Day 98; Main Study animals were dosed through Day 272 and necropsy occurred on Day 273; Recovery Study animals were dosed through Day 272 and necropsy occurred on Day 301.

Observations and Results

Mortality (2x/day)

None

Clinical Signs (Daily)

Test article-related clinical signs were limited to reddening of the eye and eyelid, as well as partial eye closure. These changes were transient, did not persist beyond Week 8 (except for reddened eyelid in female # 4508), and were not present at the time of terminal or recovery necropsy.

<u>0.04% AR-13324 Ophthalmic Solution</u>

- Male # 4002 - Partial eye closure in both eyes on Days 4 to 7, reddened eye and/or eyelid in both eyes on Days 4 and/or 11
- Female # 4508 - Reddened eyelid in the left eye on Days 250/251
- Females # 4501, 4507, and 4508 - Partial eye closure in 1 or both eyes on Days 41-52, 40, and 40/53, respectively.

0.02% AR-13324 Ophthalmic Solution

- Male # 3007 - Partial eye closure in both eyes on Day 40

Body Weights (Weekly)

No test article-related effects were apparent. Both genders were pooled in the mean summary table.

Feed Consumption (Daily; qualitatively measured)

No test article related effects

Gross Ocular Observations (Once prior to study start; after the last daily dose on Days 1-7, during Week 4, and continuing every 4 weeks thereafter until interim and terminal necropsy; Days 274-279 and weekly thereafter for recovery animals)

During the first week of dosing, findings of hyperemia, chemosis (less often), and/or discharge (rare) were noted in one or both eyes of 1 of 12 animals at 0.01% BID, 8 of 20 animals at 0.02% BID, and 15 of 20 animals at 0.04% BID. These findings were transient and mild. At Week 4, only 1 placebo-treated animal showed mild hyperemia in both eyes. All eyes of all other animals were normal at Week 4 and onward, indicating resolution of ocular irritation.

Ophthalmoscopy (Slit lamp with corneal fluorescein staining and indirect ophthalmoscopy; once prestudy; 1 hour ± 30 minutes after the last daily dose during Weeks 1, 13, 26, and 39; and during Week 42 [recovery]).

Faint (score =1) superficial corneal haze attributed to the test article in the nasal cornea was observed in both eyes of 2 mid-dose females (# 3507 and # 3510) at Week 1 (Table 4). At Week 13 and 26, the corneal haze was not present in female # 3507 but persisted in both eyes of female # 3510. The corneal haze resolved in this animal by Week 39.

At the high-dose, faint superficial corneal haze was noted in the nasal cornea of both eyes of males # 4008, 4009, and 4010, and in female # 4506; and diffuse (involving the entire cornea) superficial corneal haze (score = 1) was noted in both eyes of female # 4501 at Week 1.

At the Week 13 examinations, the corneal haze in the nasal corneas of both eyes persisted unchanged in animals # 4008, 4009, and 4010, and was also noted in male # 4005. Additionally, faint superficial corneal haze was noted in the central and nasal

corneas of both eyes in females # 4506 and 4510. The diffuse superficial corneal haze involving the entire cornea persisted unchanged in female # 4501 and had also developed in both eyes of male # 4003.

At Week 26, corneal haze persisted in animals # 4005, 4008, 4507 and 4510. The haze was fully resolved in all animals except female # 4510 at Week 39, and it was fully resolved in this animal at Week 42 (recovery period).

Table 4: Incidence of Corneal Haze in Monkeys - 9-Month Ocular Toxicity Study

Group	Monkey #	Week 1	Week 13	Week 26	Week 39	Week 42
0.02% BID (mid-dose)	M 3009	0	1 (R eye)	0	0	0
	F 3507	1	0	0	0	NEC
	F 3510	1	1	1	0	0
0.04% BID (high-dose)	M 4003	0	1	NEC	---	---
	M 4005	0	1	1	0	NEC
	M 4008	1	1	1	0	NEC
	M 4009	1	1	0	0	0
	M 4010	1	1	0	0	0
	F 4501	1	1	NEC	---	---
	F 4506	1	1	0	0	NEC
	F 4507	0	0	1	0	NEC
	F 4509	1 (L eye)	0	0	0	0
	F 4510	0	1	1	1	0

M = male; F = female; L= left; R = right; NEC = animal had undergone necropsy

The area of the cornea involved was generally 26-50% (score = 2) except for the following animals at the high dose: *female # 4501*, 76-100% (score =4) on Week 1 and 13 (necropsied at Week 13); *female # 4510*, 51-75% (score=3) on Week 13, 76-100% (score =4) on Week 26, 1-25% (score = 1) on Week 39; *male # 4003*, 76-100% (score =4) on Week 13 (necropsied at Week 13).

There was no correlative histologic finding for the corneal haze.

Fluorescein staining score was 0 for all eyes at all timepoints, indicating no damage to the corneal epithelium.

Electroretinography (Scotopic and photopic ERGs once prestudy and during Week 13, Week 39, the last week of the recovery period)

No test article-related effects were apparent. Both genders were pooled in the mean summary table.

Tonometry (Once prestudy; 1 hour ± 30 minutes after the last daily dose on Day 1, and after the last daily dose during Weeks 13, 26, and 39; during Weeks 41 and 43 [recovery])

Both genders were pooled in the mean summary table. There was a slight decrease in mean IOP in all test article-treated group in Week 1 (Day 1 at 1 hour postdose) and Week 13, compared to controls. The decrease in mean IOP values was

generally greatest in the high dose group (10.8%-20.2%); the change in this group was statistically significant at several timepoints compared to control eyes. IOP reduction is the expected pharmacological effect of the test article.

At subsequent examinations (Weeks 26, and 39, and during recovery Weeks 41 and 43) the mean IOP values for all groups (including controls) tended to be higher than at Prestudy and Weeks 1 and 13. The mean IOP values in the test article-treated groups tended to be slightly lower (7%-11%) than in the control group at Weeks 26 and 39 (statistical significance only on Week 26 in mid-dose and high-dose groups). During recovery, IOP values were generally comparable to controls.

Hematology and Coagulation (See Table 5 for evaluation timepoints)

Table 5: Clinical Pathology Evaluation Samples

Group No.	Time Point	Hematology	Coagulation	Clinical Chemistry
All animals	Week -2	X	X	X
All animals	Week -1	X	X	X
1-4	Week 13	X	X	X
1-4	Week 39	X	X	X
1, 3 and 4	Week 43	X	X	X

No. = Number; X = sample collected.

No test article-related effects were apparent. Both genders were pooled in the mean summary table.

Clinical Chemistry (See Table 5 for evaluation timepoints)

No test article-related effects were apparent. Both genders were pooled in the mean summary table.

Gross Pathology (At Day 98, 273 and 301 as shown in Table 6)

Table 6: Terminal Procedures – 9-Month Ocular Toxicity Monkey Study

Group No.	No. of Animals M	No. of Animals F	Scheduled Euthanasia Day	Necropsy Procedures: Necropsy	Necropsy Procedures: Tissue Collection	Necropsy Procedures: Organ Weights	Histology	Histopathology
1	4	4	98	X	X	X	Full Tissue	Full Tissue[a]
2	3	3					Full Tissue	Gross Lesions Select Tissues[b]
3	4	4					Full Tissue	Gross Lesions Select Tissues[b]
4	4	4					Full Tissue	Full Tissue[a]
1	4	4	273	X	X	X	Full Tissue	Full Tissue[a]
2	3	3					Full Tissue	Gross Lesions Select Tissues[b]
3	4	4					Full Tissue	Gross Lesions Select Tissues[b]
4	4	4					Full Tissue	Full Tissue[a]
1	2	2	301	X	X	X	Full Tissue	Full Tissue[a]
3	2	2					Full Tissue	Gross Lesions Select Tissues[b]
4	2	2					Full Tissue	Full Tissue[a]

No. = Number; X = procedure conducted.
[a] See Tissue Collection and Preservation table for listing of tissues.
[b] Eye with bulbar conjunctiva, optic nerve, adnexa (including meibomian gland and palpebral conjunctiva), lacrimal gland, nasal cavity, and nasopharynx.

No test article-related findings

Organ Weights (The following organs were collected: See Table 6 for timepoints)

Brain Epididymis[a] Gland, adrenal[a] Gland, pituitary Gland, prostate Gland, thyroid Heart Kidney[a]	Liver Lung Ovary[a] Spleen Testis[a] Thymus Uterus

[a] Paired organ weight

No test article-related effects. Compared to controls, one high-dose male (# 4002) had increased epididymis, prostate, testis, and thymus weight and two mid-dose and two high-dose females had increased uterus weight on Day 98 necropsy. These changes were not present at Day 273 (9 months dosing) or Day 301 (recovery) necropsy. Therefore, they were considered unrelated to treatment with the test article.

Histopathology (See Table 6)

Adequate Battery: Yes

Peer Review: No

Histological Findings: Lymphoid conjunctival hyperplasia was observed in one control (mild) and one high-dose female (mild) on Day 98 (interim necropsy), two high-dose males (min and mild) and one high-dose female (mild) on Day 273 (terminal necropsy), and one mid-dose female (min) and one high-dose male (mild) at recovery necropsy. The higher incidence at the high-dose supports a potential relationship to the test article. One high-dose female showed bilateral cystic degeneration of the ora serrata (mild) on Day 273; a relationship to the test article is uncertain. Mild atrophy of the thymus observed in one high-dose male on Day 98 necropsy was not observed in any animal at Day 273 necropsy. The applicant concluded there were no test article-related effects.

Toxicokinetics (Plasma collected from control, mid-dose and high-dose groups on Days 1, 93, and 270 at 0 hour before the 1st daily dose and 15 min and 30 min after the last daily dose; analytes includes parent test article [comprised of 2 enantiomers, AR-13324 and (b) (4)] and the specified respective metabolites of these enantiomers [AR-13503 and (b) (4)])

Plasma levels of the test article and its metabolites were below the limit of quantitation (<1 ng/mL).

Dosing Solution Analysis

The dosing solutions were within specification (b) (4)% of label claim) at release, and 1, 3 and 9 months after release (testing performed in samples stored at 5ºC/Ambient RH; at 3 months, samples stored at 5ºC/Ambient RH followed by 2 days at 25ºC /40% RH were also tested).

Study title: A 3/6-Month Ocular Toxicity Study with AR-13324 Ophthalmic Solutions in Dutch Belted Rabbits

Study no.:	AR-13324-AS15
Study report location:	EDR Module 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8-30-2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AR-13324 Ophthalmic Solution 0.01%, lot # BCL545-144B, 99.1% label claim at release testing AR-13324 Ophthalmic Solution 0.02%, lot # BCL545-144C, 99.7% label claim at release testing AR-13324 Ophthalmic Solution 0.04%, lot # BCL545-144D, 100.4% label claim at release testing **Note:** These are the same batches used in the 9-month ocular toxicity study in monkeys (Study # AR-13324-AS14).

Key Study Findings

- Clinical signs of redness of the conjunctiva and sclera and ocular discharge were observed in all groups throughout the study. The overall incidence, severity and frequency were dose dependent with findings observed sporadically in controls and low-dose groups.

- Ophthalmoscopy observations showed mild conjunctival irritation in one or both eyes during the first 3 months of dosing, consisting of redness, discharge, and/or chemosis. These findings were not observed at the Day 99 (Week 15), Day 155 (Week 23) and Day 190 (recovery) evaluations.

- At Week 13, slit lamp evaluations showed corneal opacity in one or two animals at all test article dose levels. Except at the high-dose, the finding was unilateral. The affected eyes showed punctate fluorescein uptake in the central cornea. The fluorescein uptake was minimal in severity and was limited to < 25% of stromal surface area. All eyes appeared normal at the Week 18 (dosing phase) and Week 28 (recovery) examinations.

- Microscopically, findings consisted of corneal lesions (peripheral vascularization, mixed cell inflammation, and attenuation of the overlying corneal epithelium) in 3 high-dose males, a small ulcer in one high-dose male, and minimal mixed cell

inflammation in the peripheral cornea without the vascular or epithelial changes in one high-dose female at the terminal sacrifice. There were no corneal findings noted at the interim or recovery sacrifice.

- AR-13324 was detected in one high-dose male on Week 12 at the 0.5 hour timepoint (2.09 ng/mL). For all other plasma samples, the levels were below the lower limit of quantitation (<1 ng/mL).

- Active metabolite AR-13503 was observed at low levels (1.02-1.60 ng/mL) on Day 1 in plasma samples from the mid-dose and high-dose groups at the 0.5 and/or 1 hour timepoints.

- Based on the corneal findings observed microscopically, the NOAEL was considered to be the mid dose (0.02%, BID or 7 µg/eye/dose, BID).

Methods

Doses:	See Dose Levels table below.
Frequency of dosing:	One drop/eye 2x/day (7 hour 38 min to 8 hour 26 min apart; ~ 6 hour 14 min apart on Day 142/143 only) for 182 days (6 months)
Route of administration:	Topical ocular instillation
Dose volume:	~35 µL/drop
Formulation/Vehicle:	AR-13324 Ophthalmic Solution Placebo
Species/Strain:	Dutch Belted rabbits
Number/Sex/Group:	See Dose Levels table below.
Age:	~7 months old
Weight:	1.7 to 2.4 kilograms
Satellite groups:	None
Unique study design:	The study had a 2-week recovery period.

In Quality section 2.3.P.5 Control of Drug Product, the applicant indicates that the dosing solutions in nonclinical batches used in this study were spiked with [redacted] (b) (4) at 5%, AR-13324 active metabolite at 0.5%, and impurities [redacted] (b) (4) at 0.5%.
This information was not acknowledged in study report but the Certificates of Analysis confirm the statement [redacted] (b) (4)

The placebo solution was not spiked with these impurities, per certificate of analyses.

Deviation from study protocol: None with an impact on the validity of the study

Dose Levels

Group	Total Daily Dose* (mg/animal/day)	Concentration (% w/v)	Approximate Dose Volume/Dose (µl/dose)	Approximate Total Dose Volume* (µl/day)	Number of Animals**	
					Male	Female
1. AR-13324 Ophthalmic Solution Placebo	0	0.0	35/eye	140	11	11
2. 0.01% AR-13324 Ophthalmic Solution	0.01	0.01	35/eye	140	9	9
3. 0.02% AR-13324 Ophthalmic Solution	0.03	0.02	35/eye	140	9	9
4. 0.04% AR-13324 Ophthalmic Solution	0.06	0.04	35/eye	140	11	11

*Both eyes were dosed b.i.d. (one drop/eye/dose) during the dosing phase. The dose volumes and mg/kg dosages were based on the presumption that the average drop was approximately 35 µL, according to the Sponsor.

** A targeted 4 animals/sex/group were necropsied on Day 92. A targeted 5 animals/sex/group were necropsied on Day 183. A targeted 2 animals/sex/group in Groups 1 and 4 were necropsied on Day 197.

Observations and Results

Mortality (2x/day)

None treatment related

Clinical Signs (Daily)

Findings indicative of ocular irritation were observed throughout the study. Redness of the conjunctiva and sclera and ocular discharge were observed in all groups, including the placebo control. The overall incidence, severity and frequency were dose dependent with findings being observed sporadically in controls and low-dose groups. The findings were not present during the recovery period.

- Redness of the conjunctiva and/or sclera was noted in controls (4 out of 11 males; 5 out of 11 females), low-dose (7 out of 9 males; 6 out of 9 females), mid-dose (8 out of 9 males; 8 out of 9 females) and high-dose (11 out of 11 males; 11 out of 11 females) groups. The severity of redness was generally mild in controls and low-dose, and mild to moderate in the mid-dose and high-dose group.

- Ocular discharge was noted in controls (1 out of 11 females), low dose (1 out of 9 females), mid dose (3 out of 9 males; 5 out of 9 females) and high dose (11 out of 11 males; 10 out of 11 females). The severity was small amounts in female controls and at the female low dose, small to medium amounts at the mid dose, and small to large amounts at the high dose.

- Crusted eyes were noted in two male and two females at the high dose; this sign was transient and generally resolved within 1-2 days.

Body Weights (Weekly)

No test article-related effects

Feed Consumption (Daily)

No test article-related effects

Ocular Observations (Once prior to treatment initiation, during Weeks 2, 6, 11, 15 and 23 of the dosing phase and during the final week of the recovery phase; Draize score) -

Conjunctival irritation was observed in one or both eyes during the first three months of dosing, consisting of redness, discharge, and/or chemosis. These findings were initially observed on Day 8 at the high dose and on Day 36 at the mid dose. Individual findings in affected animals are shown below (Draize score given within the parenthesis):

Table 7: Individual Ocular Observations (Draize Scores)- 3/6-Month Ocular Toxicity Study in Rabbits

Finding	Day 8 [Week 2]	Day 36 [Week 6]	Day 71 [Week 11]
Redness			
Mid dose	Not observed	Male # 1522 (score 1) Female # 1566 (score 1)	Female # 1566 (score 1)
High dose	Male #1538 (score 1) Male #1539 (score 1) Female # 1572 (score 1)	Male # 1530 (score 1) Male # 1532 (score 1) Male # 1536 (score 1) Male # 1538 (score 1) Male # 1539 (score 1) Female # 1573 (score 1)	Male # 1532 (score 1) Male # 1539 (score 1) Male # 1540 (score 1) Female # 1573 (score 1)
Chemosis			
Mid dose	Not observed	Not observed	Not observed
High dose	Male #1531 (score 1)	Female # 1573 (score 2)	Not observed
Discharge			
Mid dose	Not observed	Not observed	Not observed
High dose	Male # 1531 (score 1) Male # 1538 (score 1) Male # 1539 (score 1)	Female # 1573 (score 3 – right eye; score 1 – left eye)	Male # 1537 (score 1) Female # 1575 (score 1) Female # 1576 (score 1)

Data represent 9 rabbits/sex at the mid dose and 11 rabbits/sex at the high dose.

As seen in Table 7, all findings were of mild severity (score 1), except for one high-dose female with score 2 chemosis and score 3 discharge in the right eye on Day 36. These findings resolved in this female by Day 71. Except for this incidence, findings were indicative of minor irritation. No findings were observed at the Day 99 (Week 15), Day 155 (Week 23) and Day 190 (recovery) evaluations.

Ophthalmoscopy (Slit lamp with fluorescein staining and indirect ophthalmoscopy; before treatment initiation and following the final daily dose during Weeks 1, 4, 9, 13, 18 and 26 of the dosing phase and during the final week of recovery; McDonald and Shadduck scoring system) -

Unilateral conjunctivitis was observed in 4 females (# 1570, 1571, 1572 and 1573) at the high dose on Day 5/3. McDonald and Shadduck scores of 1 were recorded for conjunctival congestion and swelling in one or both eyes in these 4 high-dose females. These eyes were normal in subsequent evaluations.

At Week 1, bilateral conjunctival congestion (score 1) was noted in 1 high-dose male (# 1536) and an additional 3 high-dose females (# 1574, 1575 and 1577); unilateral conjunctival congestion was noted in female # 1578.

At Week 4, bilateral conjunctival congestion and discharge of score 1 were only noted in high-dose male # 1539. Both eyes were normal in all other animals showing findings at earlier time points.

These findings were not present at subsequent examinations.

At Week 13, McDonald and Shadduck scores of 1 were recorded for corneal opacity, % involvement (< 25% of stromal area), and fluorescein staining in one eye in 1 female (# 1557) at the low dose, and 1 male (# 1525) and 1 female (# 1566) at the mid dose, and in both eyes in 1 female at the high dose (# 1579). The study report describes the fluorescein staining in these animals as punctate fluorescein uptake in the central cornea.

At Week 18 all eyes appeared normal. At Week 26, bilateral discharge (score 2) was noted in one mid-dose female (# 1568) out of 5 females examined.

At Week 28 (recovery), both eyes of both animals/sex in control and high-dose groups were normal.

Electroretinography (ERG) (Scotopic and photopic ERGs and visual evoked response/potential [VER/VEP] prior to treatment initiation and during Week 13 and the final 2 weeks of dosing) -

No test article-related effects were apparent in ERG evaluations. The ERG reports stated that flicker and VEP responses were not quantitatively assessed, but they were present in all rabbits tested at all time-points.

Tonometry (Weeks 1, 5, 9, 13, 18 and 26 of the dosing phase and during the final week of the recovery period)

In males, there was a tendency towards slightly lower mean IOP values in all test article-treated groups compared to concurrent control. The decrease was statistically significant only in a few instances: right eye on Day 4 in mid-dose and high-dose males,

and in left eye on Day 7, Day 29 and Day 182 in high-dose males. Individual animal listings showed that overall IOP values were within concurrent control range or baseline range.

Hematology and Coagulation (Prior to dosing initiation and during Week 13, Weeks 27, and 29)

No test article-related effects

Statistically significant changes were noted in PT and APTT values in high-dose females during recovery (shown below).

Group #		PT (sec)	APTT (sec)
Control	(n)	2	2
	Means	8.25	16.20
	Sdevs	0.354	0.707
4	(n)	2	2
	Means	9.65*D	10.75+D
	Sdevs	0.212	0.354

Values were within performing laboratory historical control range (see below) for APTT, but above historical control range for PT. However, it is difficult to attribute PT changes to the test article-related based on normal values observed during treatment.

FEMALE COAGULATION DATA

DIAGNOSTICA STAGO STA COMPACT

COAGULATION TEST	N	MEAN	S.D.	LOWER–UPPER LIMIT
PROTIME (PT) sec.	284	7.5	0.44	6.6 – 8.4
ACTIVATED PARTIAL THROMBOPLASTIN-TIME (APTT) sec.	287	13.7	2.04	9.6 – 17.8

Clinical Chemistry (Prior to dosing initiation and during Week 13, Weeks 27, and 29)

No test article-related effects

Gross Pathology (Day 92 [interim sacrifice], Day 183 [terminal sacrifice] and Day 197 [recovery sacrifice])

No test article-related findings

Organ Weights – (Day 92 [interim sacrifice], Day 183 [terminal sacrifice] and Day 197 [recovery sacrifice]; organs listed below for all groups):

Organs Weighed	
Adrenals	Testes
Brain	Ovaries
Heart	Spleen
Kidneys	Thyroids/parathyroids
Liver	

No test article-related changes

Histopathology (Full battery of systemic tissues for control and high-dose groups; ocular tissues for all groups)

Adequate Battery - Yes

Peer Review - No

Histological Findings- At the terminal sacrifice on Day 183, 3 high-dose males had corneal lesions consisting of peripheral vascularization, mixed cell inflammation, and attenuation of the overlying corneal epithelium, with a small ulcer in one male (# 1535). One high dose female (# 1575) had minimal mixed cell inflammation in the peripheral cornea without the vascular or epithelial changes. As noted in the pathology report, this likely represents a test article-related effect.

There were no corneal findings noted at the interim or recovery sacrifice.

Findings observed in control and test article-treated groups included focal hypertrophy of the retinal pigmented epithelium (adjacent to the optic disc), mononuclear cell infiltrate in the limbus, acanthosis and perifollicular cell inflammation in the eyelids, chronic inflammation in the Harderian gland, and heterophil infiltrate in the nictitating membrane mucosa and palpebral conjunctiva. The severity was minimal to mild. These were considered incidental background findings.

Minimal corneal dystrophy was also seen in one high-dose male. The pathology report indicates that this is a known incidental finding in Dutch Belted rabbits.

Toxicokinetics (Plasma collected on Days 1, Week 12 and Week 26 at 0 hour before the 1st daily dose and 30 min and 1 hour after the last daily dose; analytes includes parent test article [comprised of 2 enantiomers, AR-13324 and (b) (4)] and the specified respective metabolites of these enantiomers [AR-13503 and (b) (4)])

AR-13324 was detected in one high-dose male on Week 12 at the 0.5 hour timepoint at a plasma level of 2.09 ng/mL. For all other plasma samples, the levels were below the lower limit of quantitation (<1 ng/mL).

Metabolite AR-13503 was detected in five control samples from 6 different animals on Day 1 at the 0.5 or 1 hour timepoints. The plasma levels ranged from 1.23-1.48 ng/mL, i.e., they were near the lower limit of quantitation (<1 ng/mL). Metabolite AR-13503 was also observed at similar levels (1.02-1.60 ng/mL) on Day 1 in plasma samples from the mid-dose and high-dose groups at the 0.5 or 1 hour timepoints (except one male with detectable levels at both timepoints).

The (b) (4) impurity of AR-13324, coded (b) (4) or its metabolite (b) (4) was not detected in any of the study animals.

The bioanalytical laboratory conducted an investigation to determine possible causes of AR-13503 present in control samples. The exact cause could not be determined. Based on the available data, the Principal Investigator determined that sample contamination during bioanalysis or endogenous interference were unlikely. However, the nature of the measurements is unclear. Given the low levels observed and lack of any systemic toxicity, this limitation is not considered of major impact in the interpretation of the data.

Dosing Solution Analysis

The dosing solutions were within specification (b) (4) % of label claim) at release and at 1, 3, and 6 months after release (testing performed in samples stored at 5°C/Ambient RH and in samples stored for 3 month at 5°C/Ambient RH followed by 2 days at 25°C /40% RH were also tested).

The following studies were reviewed under the initial review of IND 113064 (7-day studies) and SD # 9 (28-day studies). The following summaries are consistent with the information presented in prior IND 113064 nonclinical reviews:

A 7-Day Ocular Toxicity Study of AR-13324 Ophthalmic Solutions in Dutch Belted Rabbits with a 7-Day Recovery (Study # AR-13324-AS05; GLP; Module 4.2.3.2) – Treatment groups included 0 (Placebo Solution) QID, 0.04% QD, 0.08% QD, and 0.04% QID (30 µL/instillation). Both eyes were treated. The lots of AR-13324 Ophthalmic Solution used contained (b) (4) % chiral impurity.

Key Study Findings

- Topical ocular administration of AR-13324 Ophthalmic Solution 0.04% QID resulted in severe ocular irritation during the first few days of treatment. As the dosing phase progressed, the irritation lessened in both severity and incidence.
- Corneal epithelial damage (fluorescein staining) was noted at 0.04% QID. The finding resolved during the recovery phase.

- Histopathology evaluations showed minimal or slight cellular infiltrates (mononuclear, heterophil, and/or eosinophil) in the bulbar and palpebral conjunctiva, eyelids and Harderian glands. The Harderian glands also showed minimal degeneration of the acinar cells. These findings, however, were also observed in control eyes and did not show a clear dose response or increased incidence and/or severity to suggest a relationship to the test article.
- Based on the severe ocular irritation and high incidence of corneal staining at 0.04% QID, the NOAEL was 0.08% QD.
- AR-13324 systemic exposure across doses was low (C_{max} ≤ 1.58 ng/mL and $AUC_{0\text{-}18hr}$ ≤ 5.3 ng•hr/mL).
    - In this study, AR-13324 represents the combined values of AR-13324 plus primary metabolite AR-13503.

A 7-Day Ocular Toxicity Study of AR-13324 in Cynomolgus Monkeys with a 7-Day Recovery Period (Study # AR-13324-AS06; GLP; Module 4.2.3.2) - Treatment groups included 0 (Placebo Solution), 0.01%, 0.04%, and 0.12% QID. Both eyes were treated (30 µL/instillation). The lots of AR-13324 Ophthalmic Solution used contained (b) (4)% chiral impurity.

Key Study Findings

- Findings indicative of irritation including reddened conjunctiva, eye discharge, reddened sclera, and/or swollen conjunctiva were noted between Day 1 and Day 7 ≥ at 0.04% QID.
- Corneal changes characterized by corneal cloudiness (Grade 1-2), with or without corneal edema, were observed at ≥0.04% QID.
- The corneal cloudiness persisted throughout the recovery period. All other changes were not observed in recovery animals.
- Microscopic test article-associated findings were observed in the cornea (minimal to moderate hypertrophy/hyperplasia/edema/ apoptosis), eyelids (minimal to mild hypertrophy/hyperplasia of the conjunctival epithelium/inflammatory cell subconjunctival infiltrates), and nasolacrimal ducts (mild mononuclear cell or neutrophil infiltrates/edema).
- At recovery, microscopic findings of corneal hypertrophy/hyperplasia and apoptosis as well as hypertrophy and hyperplasia in the eyelids were still present at 0.12% QID (only test article dose evaluated). The findings in the nasolacrimal ducts were also present but with similar incidence/severity as controls. The minimal to mild hypertrophy and hyperplasia in the eyelids did not change substantially during the recovery period; the severity/incidence of inflammatory infiltrates was similar to that observed in controls.
- The applicant considered the NOAEL was 0.01% QID. Based on the minimal severity of the findings at this dose, the determination was considered acceptable.
- Systemic exposure of AR-13324 was not detectable at ≤0.04% QID. At the high dose, levels ≤0.3 ng/mL were detected on Day 1. The levels were below the limit

of quantitation (0.1 ng/mL) on Day 7 (except for one male with levels of 0.101 ng/mL).

A 7-Day Ocular Toxicity Study of AR-13324 in Cynomolgus Monkeys (Study # AR-13324-AS07 (Study # AR-13324-AS07 GLP; Module 4.2.3.2) - This was a follow up study to Study # AR-13324-AS06 to explore the ocular tolerance at doses of 0.02% BID, 0.04% QD and BID, and 0.08% QD (30 µL/instillation).

Key Study Findings

- Gross ocular observations and ophthalmologic evaluations showed indications of irritation (conjunctival congestion and swelling) noted at all dose levels. These were generally mild (Grade 1 or 2) on Day 1. The severity (Grade 1) and incidence decrease on Day 7.
- Microscopically, the main findings were corneal epithelial hyperplasia/ hypertrophy, hypertrophy/hyperplasia of the conjunctival epithelium and/or epidermis of the eyelids at all doses. These findings were of minimal to mild severity.
- AR-13324 plasma levels were below the limit of quantitation (0.10 ng/mL) for all animals except those at 0.08% QD.
- The applicant selected the NOAEL as 24 µg/eye/day delivered as 0.04% BID or 0.08% QD. This reviewer believes that based on the minimal findings at the lower dose, a NOAEL of 12 µg/eye/day delivered as 0.02% BID or 0.04% QD may be more appropriate.
- Overall, the findings were of lower severity than those seen in Study # AR-13324-AS06 at doses ≥0.04% QID.

A 28-Day GLP Ocular Toxicity Study of AR-13324 Ophthalmic Solution in Dutch-Belted Rabbits with a 14-Day Recovery Period (Study # AR-13324-AS11; GLP; Module 4.2.3.2) - Dutch-Belted rabbits (8/sex/group for control and high-dose group; 3/sex/group for low- and mid-dose groups) were treated with vehicle or AR-13324 Ophthalmic Solution 0.02, 0.04, or 0.06% BID in both eyes for 28 days. The test article was administered by topical ocular administration in a drop volume of ~30 µL. Three rabbits/sex/group in control and high-dose groups were retained for a 2-week recovery period. The dosing solutions contained enantiomer AR-13324 at a level of 5.0-5.1%.

Evaluations consisted of mortality, clinical signs, body weights, food consumption, ocular irritation (Draize scale), ophthalmology, intraocular pressure, electroretinograms, hematology/coagulation, clinical chemistry, and TK. Gross necropsy, selected organs weight (adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, thyroid/parathyroids), and histopathology of ocular and systemic tissues were evaluated in control and high-dose groups at the end of the treatment and recovery periods; ocular histopathology was evaluated in low and mid-dose groups at the end of the treatment period.

Key Study Findings:

Eye irritation (Draize scoring) - Conjunctival redness of minimal severity was observed at ≥0.04% BID AR-13324 Ophthalmic Solution. The redness was first noted on Day 1 but showed diminishing incidence with continuous dosing. Minor conjunctival discharge was sporadically noted also at ≥0.04% BID. The eyes were normal throughout recovery.

Ophthalmology (McDonald & Shadduck scoring) – On Day 1, test article-related findings included unilateral ventral corneal ulcer in one low-dose female, unilateral punctate corneal opacity in one low-dose female, and focal unilateral corneal opacities in one low-dose male and one high-dose male. One control male also had a focal unilateral corneal ulcer on Day 1. Conjunctival congestion, swelling, and/or discharge were present in 1 low-dose female, 1 mid-dose male, and 4 males and 8 females at the high-dose.

On Day 7, the only finding observed was conjunctival congestion in 4 males and 2 females at the high dose. On Day 14, conjunctival congestion, swelling, and/or discharge were observed in 4 high-dose females and congestion was observed in 1 mid- and 1 high-dose male. Two mid-dose females had bilateral punctuate corneal ulcers and one high-dose female had a unilateral punctuate corneal ulcer.

On Day 28, conjunctival congestion, and swelling were noted in 1 mid-dose and 6 high-dose females, congestion was noted in 2 mid-dose and 1 high-dose male, and discharge was noted in 1 high-dose female. These findings were of slight severity (score 1) and mostly bilateral.

Corneal findings were not present on Day 28 (last day of dosing). The % corneal involvement score was always 1 (1-25% stromal cloudiness). The study report acknowledged that an ophthalmic examination was inadvertently not performed during recovery.

Organ weights - High-dose females had a statistically significant increase in mean ovary weight on Day 29 (45%). There was no microscopic correlate.

Histopathology – Slight degeneration/erosion of the cornea was observed in one mid-dose male, 2 high-dose males, and 1 high-dose female. The applicant further described that none of these eyes had progressed to an area of denuded corneal epithelium, which would have been diagnosed as corneal ulceration (exposure/damage of underlying sub-epithelial basement membrane). In these animals, the epithelial layer was somewhat disorganized and the overall epithelial layer was slightly thinner but the subepithelial basement membrane was not exposed. The finding was not observed in recovery animals.

TK: Plasma samples were analyzed for levels of parent test article (comprise of two enantiomers, AR-13324 and [redacted]) and metabolites AR-13503 [redacted] on Days 1, 7, and 28 (0-2 hours for controls, 0.5-1 hours for low-dose, 0.5-16 hours for and mid-dose animals high-dose animals after the last daily dose). One mid-dose male had AR-13324 levels of 1.13 ng/mL at 0.5 hour postdose on Day 7; one high-dose female had levels of 1.03 ng/mL at 0.5 hour and 11.8 ng/mL at 16 hours postdose on Day 1. For all other animals, the levels of AR-13324 and all 3 other analytes were below the lower quantitation limit (1 ng/mL). As a note, however, most samples (including controls) were hemolyzed.

Other parameters: There were no test article-related effects all other parameters evaluated.

NOAEL: The applicant selected the low dose (0.02% BID) as the NOAEL. This reviewer concurs based on the observed conjunctivitis, corneal opacities, and slight degeneration/erosion in the cornea at ≥0.04% BID.

A 1-Month Ocular Toxicity Study of AR-13324 in Cynomolgus Monkeys with at 2-Week Recovery Period (Study # AR-13324-AS12; GLP; Module 4.2.3.2) - Cynomolgus monkeys (5/sex/group for control and high-dose group; 3/sex/group for low- and mid-dose groups) were treated with vehicle or AR-13324 Ophthalmic Solution 0.02, 0.04, or 0.06% BID (0, 12, 24, and 36 µg/day, respectively) in both eyes for 35 days. The test article was administered by topical ocular administration in a drop volume of ~30 µL. Three monkeys/sex/group in control and high-dose groups were retained for a 2-week recovery period. The dosing solutions contained enantiomer AR-13324 at a level of 5.0-5.1%.

Parameters evaluated included mortality, clinical signs, body weights, food consumption, slit-lamp biomicroscopy (with fluorescein staining), indirect ophthalmoscopy, intraocular pressure, electroretinograms, hematology/coagulation, clinical chemistry, and toxicokinetics. Gross necropsy, selected organs weight (adrenals, brain, epididymis, heart, kidneys, liver, lung, ovaries, pituitary, prostate, spleen, testis, thymus, thyroid, and uterus) and histopathology (ocular and systemic tissues were evaluated in control and high-dose groups at the end of the treatment and recovery periods; ocular histopathology was evaluated in low and mid-dose groups at the end of the treatment period; nasolacrimal tissue was evaluated in all groups).

Key Study Findings:

Clinical signs: Redness around the eye, periorbital swelling, and/or ptosis were observed at ≥0.04% BID. These findings were transient and/or sporadic and did not persist with continuing dosing.

Ophthalmology (Modified McDonald & Shadduck scoring) – Corneal haze was noted in both eyes in 2 of 6 mid-dose animals and all 10 high-dose animals. The findings was first noted on Day 28/29 in mid-dose animals and on Day 7/8 in high-dose animals.

The finding persisted throughout the dosing period but resolved during the recovery period. The corneal haze (score 1) was characterized by diffuse haze that had the appearance of multiple, very fine, pinpoint depositions or droplets in the superficial cornea. The area of the cornea involved ranged from 26-100% (scores of 2-4).

Tonometry – Decreased mean IOP (pooled male and female data) was observed at all AR-13324 Ophthalmic Solution dose levels compared to prestudy and concomitant control values. In general, the mean values at the high dose tended to show a greater effect. IOP decreases were evident within 1 hour following the last dose (1st time point evaluated). The individual animal listing showed decreases of up to 64% (compared to prestudy values). Not all animals within each dose group were affected. In general, the magnitude of the effect diminished as treatment progressed. IOP returned to normal levels at the end of recovery.

Histopathology – Minimal unilateral hyperplasia was noted in the corneal epithelium of one male and one female at the high dose. All animals, including controls, showed lymphocytic infiltration of the conjunctiva in both eyes. The severity was minimal to moderate. Particularly in males, the severity tended to be higher compared to control; in females, there was not a clear difference. A higher incidence of minimal to mild lymphocytic infiltration of the lacrimal gland was observed in males at all test article doses and in high-dose females, compared to controls. At recovery, lymphocytic infiltration of the conjunctiva was still present in control and high-dose animals. The severity was minimal to mild except for moderate lymphocytic infiltration of the conjunctiva in both eyes of one high-dose male and the right eye of one high-dose female.

Toxicokinetics - Plasma samples were analyzed for levels of parent test article (comprise of two enantiomers, AR-13324 and (b) (4)) and metabolites AR-13503 (b) (4) Blood was collected at 0.25 and 0.5 hours after the last daily dose on Days 2 and 30 for control, low-dose, and mid-dose animals; and at 0.25, 0.5, 1, 2 and 4 hours after the last daily dose on Days 2 and 30 for high-dose animals. At all dose levels, the plasma concentrations of AR-13324 and all 3 other analytes were below the lower quantitation limit (1 ng/mL).

Other parameters: There were no test article-related effects in all other parameters evaluated.

NOAEL: Based on the presence of corneal cloudiness/haze at the mid-dose and high-dose, the applicant considered the NOAEL was the low dose of 0.02% BID. This reviewer concurred with the NOAEL selection.

Systemic Route Repeat-Dose Toxicity Studies

A 7-Day GLP Intravenous Toxicity Study of AR-13324 in Rats with a 7-Day Recovery (Study # AR-13324-AS03; **GLP; Module 4.2.3.2) -** Sprague-Dawley rats (10/sex/group) received AR-13324 as an IV bolus injection at doses of 0, 1, 3, and 10 mg/kg. Assessment of CNS effects (see Section 4.3 Safety Pharmacology) and micronucleus evaluation (see Section 7. Genetic Toxicology) were also conducted in this study.

Key Study Findings

- Clinical signs were noted at 10 mg/kg/day, including primarily red skin on various body surfaces and abnormal appearance of the injection site (tail). The tails continued to have abnormal appearance through the end of the recovery period.
- Hematology evaluations showed increases/decreases in erythrocyte parameters mostly at ≥ 3 mg/kg and in several leukocyte parameters at 10 mg/kg, abnormal erythrocyte morphology at 10 mg/kg, and increased in mean prothrombin time at 10 mg/kg. Most findings resolved during recovery.
- Clinical Chemistry showed changes included increased total bilirubin at ≥3 mg/kg and globulin at 10 mg/kg, and decreased total protein, albumin, and albumin-to-globulin ratio at 10 mg/kg. These findings resolved during recovery.
- Microscopic evaluations showed injection site alterations at 10 mg/kg in males and ≥3 mg/kg in females (mostly of minimal to slight severity), increased incidence of hepatic extramedullary hematopoiesis (min to slight) at ≥ 3 mg/kg in males, a decrease in the background finding of hepatocellular vacuolation in females at 10 mg/kg, increased incidence of minimal glandular dilatation of the stomach at 10 mg/kg, increased incidence/severity of dilatation of the uterus at 10 mg/kg, and increased incidence of minimal fibrosis in the lungs at 10 mg/kg. Recovery animals were not evaluated microscopically.
- The NOAEL was determined to be 1 mg/kg/day (C_{max} of 14 ng/mL and $AUC_{0\text{-}24hr}$ of 62 ng•hr/mL on Day 7).

A 7-Day GLP Intravenous Toxicity Study of AR-13324 in beagle Dogs (Study # AR-13324-AS04; GLP; Module 4.2.3.2) – Beagle dogs (3/sex/group) received AR-13324 as an IV infusion at doses of 0, 1, 12.5/3, and 25/6 mg/kg.

Key Study Findings

- Animals at 12.5 and 25 mg/kg were euthanized due to moribund conditions. Because of these mortalities, the mid- and high-dose levels were reduced to 3 and 6 mg/kg, respectively.
- Swelling of the forelimbs at the injection site, red skin (ears, forelimbs, and abdomen) and red eyes/sclera were observed at all doses. These signs resolved completely for low dose females (normal at Days 7 and 8). Low dose males continued to exhibit forelimb swelling on Day 8.

- Red chest, red sclera, red muzzle, loose feces, decreased activity, labored respiration, and clear ocular discharge were observed at the mid and high dose. Additional signs observed at the high dose included green, frothy or food particle emesis, continuous, noisy vocalization, red neck, abnormal stance, and abnormal gait.
- Decreases in erythrocyte parameters were observed at ≥3 mg/kg. Additional findings in moribund animals included abnormal erythrocyte morphology, decreased activated partial thromboplastin time, and alterations in leukocyte parameters.
- Other potential target organs identified included the kidneys, lungs, heart, gallbladder, adrenal gland, bone marrow, lymph nodes, thymus, urinary bladder and liver (Table 8). Vasculitis and/or hemorrhage were observed microscopically in some of these organs.

Table 8: Microscopic Findings in the 7-Day Repeat-Dose IV Study in Dogs

Organ	Microscopic Finding Description
Kidney	Granulocytic infiltration and granular and hyaline tubular casts in high-dose moribund animals (minimal to slight); tubular basophilia and chronic inflammation in scheduled sacrifice high-dose females (minimal to moderate)
Lungs	Alveolar edema (slight to severe), hemorrhage (minimal to marked), and/or granulocytic infiltration (minimal to moderate) in the moribund animals; granulocytic infiltration in one mid- and high-dose scheduled sacrifice female (slight to moderate) and mid-dose male (slight); alveolar edema in the scheduled sacrifice mid-dose male (slight); alveolar edema (moderate) in one control female
Heart	Cardiac hemorrhage, congestion, and/or vasculitis in the moribund animals (minimal to slight); epicardial hemorrhage in scheduled sacrifice high-dose females (minimal to slight)
Gallbladder	Biliary calculi at the high dose in moribund animals and one scheduled-sacrifice female (minimal to slight); vasculitis in one high dose moribund male (minimal)
Adrenal gland	Granulocytic infiltration in the cortex in high dose moribund animals (minimal to slight)
Bone marrow	Depletion in moribund animals (minimal to slight)
Lymph nodes (mandibular/mesenteric)	Congestion in moribund animals at the high dose (minimal to slight) and one control male (slight)
Thymus	Involution in moribund animals at the high dose and one mid- and high-dose scheduled sacrifice female (minimal to slight); tingible body macrophages in moribund animals (slight to moderate)
Urinary bladder	Vasculitis in one high dose schedule sacrifice female (moderate)
Liver	Multifocal oval cell hyperplasia (minimal) in 2 low dose males – The study report indicates that although this lesion was confined to the low-dose level, it was not considered to be a typical background finding in the dog. Considering its unusual nature for the strain and age of the test species, it could be an early indication of liver damage due to test article administration.

- A series of clinical chemistry changes reflecting effects in these organs were observed (decreases in creatinine, phosphorous, potassium, calcium, albumin, and albumin/globulin ratio, and increases in total bilirubin, ALP, and ALT).

- The NOAEL was 1 mg/kg/day (C_{max} of 43 ng/mL and AUC_{0-24hr} of 264 ng•hr/mL on Day 7).

A 28-Day GLP Intravenous Toxicity Study of AR-13324 in Rats with a 14-Day Recovery (Study # AR-13324-AS09; GLP; Module 4.2.3.2) – Sprague-Dawley rats were treated with vehicle (0.9% sodium chloride) or AR-13324 at dose levels (based on free base form) ranging from 0.04-0.07 mg/kg/day (low dose), 0.16-0.21 mg/kg/day (mid-dose), and 0.59-0.74 mg/kg/day (high-dose) for 28 days. A total of 15 rats/sex/group were used in the control and high-dose groups; 10 rats/sex/group were used in the low-dose and mid-dose group. An additional 9 rats/sex/test article-treated group were used for toxicokinetics evaluation. The test article was administered by intravenous bolus injection (10 mL/kg dose volume) in the lateral tail vein. Five rats/sex/group in control and high-dose groups were retained for a 2-week recovery period. Animals were 7 weeks old at the start of dosing with body weight ranging from 220-263 g for males and 167-202 g for females. The stock dosing solution was a mixture of AR-13324 (lot # 0433385) and (b) (4) (lot # 0435347). The final (b) (4) content was (b) (4) (b) (4)%.

Parameters evaluated included mortality, clinical signs, body weights, food consumption, ophthalmoscopy, hematology/coagulation, clinical chemistry, gross necropsy, and selected organ weights (adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, thyroid/parathyroids). Full histopathology of systemic tissues was evaluated in control and high-dose groups at the end of the treatment period; liver and injection sites were evaluated in low and mid-dose groups at the end of the treatment period.

Dosing solutions initially met acceptance criteria (b) (4)%) but gradually dropped below 90% of nominal concentration as the study progressed. At the end of study, dosing solutions were 63.4, 76.1, and 84.1% of nominal concentration at 0.007, 0.021, and 0.07 mg free base/mL, respectively. Based on these results, the doses listed above represent the actual concentrations measured.

Main Results:

Mortalities: Mortalities were observed in control (2 males and 2 females) and low-dose (1 female) groups from the main animals, and in low-dose (1 male) and high-dose (1 female) groups from the TK animals. The mortalities occurred during dosing or 1-2 hours after dose administration. These deaths were considered related to dosing procedures.

Histopathology: Injection site reactions were observed in control and test article-treated groups without a dose relationship. In males, these injection site reactions included fibrosis (2 control and 1 low dose), crust formation (1 control and 2 low dose), and acanthosis (3 control and 1 low dose). In females, injection site reactions included thrombosis (1 low dose and 1 high dose), mixed cell infiltration (1 control and 1 high

dose), and fibrosis (2 controls and 1 mid dose). The severity ranged from minimal to slight. Based on incidence and severity, these findings appear related to the injection procedure rather than related to test article or vehicle (i.e., if they were vehicle mediated, they would be expected to be observed in all groups).

Other parameters: There were no test article-related adverse findings.

TK: Exposure of AR-13324 free base increased with dose dependent but the increase was greater than dose proportional. The half-life values ranged from ~0.5-4 hours. There was no accumulation with repeated dosing for 28 days. However, as noted above, the dosing solution concentrations gradually dropped as the study progressed. This may have had an impact on drug accumulation. No sex-related differences were apparent. The table below shows the mean C_{max} and $AUC_{0\text{-}24hrs}$ values observed on Day 28:

Table 9: TK Parameters: 28-Day GLP Intravenous Toxicity Study in Rats

Dose (mg/kg/day)*	C_{max} (ng/mL)		$AUC_{0\text{-}24hr}$ (ng•hr/mL)	
	Male	Female	Male	Female
0.07	4.06	4.39	3.62	4.24
0.21	18.2	12.6	24.28	19.46
0.7	64.5	57.0	125.79	121.54

*Nominal dose

(b) (4) was present in the plasma samples of mid-dose (C_{max} ≤ 0.680 ng/mL; $AUC_{0\text{-}24hr}$ ≤ 0.2268 ng•hr/mL) or high-dose (C_{max} ≤ 3.12 ng/mL; $AUC_{0\text{-}24hr}$ ≤ 2.47 ng•hr/mL) animals at the 0.167 and/or 0.5 hour timepoints on Days 1 and 28. No accumulation was apparent with repeated dosing. The study report states that the ratios of (b) (4) AUC_{last} and AR-13324 AUC_{last} (0.011-0.017) or (b) (4) C_{max} and AR-13324 C_{max} (0.035-0.048) are consistent with a chiral AR-13324 purity of >95%.

AR-13324 free base (b) (4) metabolized by ester hydrolysis to AR-13503 (b) (4) AR-13503 was detected in mid-dose high-dose animals on Days 1 and 28. The half-life values ranged from 0.45-9 hours (could only be calculated for the high dose). The C_{max} and $AUC_{0\text{-}last}$ were ≤1.00 ng/mL and 0.333 ng•hr/mL at the mid dose and 7.00 ng/mL and 6.93 ng•hr/mL at the high dose, respectively. (b) (4) was not detected.

NOAEL: The applicant selected the highest dose as the NOAEL (i.e., nominally 0.7 mg AR-13324 free base/kg/day; 0.59-0.74 mg/kg/day based on dose formulation analysis). This reviewer concurs.

A 28-Day GLP Intravenous Toxicity Study of AR-13324 in Beagle Dogs with a 14-Day Recovery (Study # AR-13324-AS10; GLP; Module 4.2.3.2) – Beagle dogs were treated with vehicle (0.9% sodium chloride) or AR-13324 at dose levels (based on free base form) ranging from 0.04-0.05 mg/kg/day (low dose), 0.15-0.18 mg/kg/day (mid-

dose), and 0.53-0.67 mg/kg/day (high-dose) for 28 days. A total of 5 dogs/sex/group were used in the control and high-dose groups; 3 dogs/sex/group were used in the low-dose and mid-dose group. The test article was administered by intravenous injection (10 mL/kg dose volume) in the cephalic (primary route) or saphenous vein (used only when the injection could not be administered via the cephalic vein). Two dogs/sex/group in control and high-dose groups were kept for a 2-week recovery period. Animals were 5 months old at the start of dosing with body weights ranging from 5.6-8.3 kg. The stock dosing solution was a mixture of AR-13324 (lot # 0433385) and (b) (4) (lot # 0435347). The final (b) (4) content was (b) (4) %.

Parameters evaluated included mortality, clinical signs, body weights, food consumption, ophthalmoscopy, intraocular pressure, electrocardiology, hematology/ coagulation, clinical chemistry, urinalysis, gross necropsy, and selected organ weights (adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, thyroid/parathyroids). Full histopathology of systemic tissues was evaluated in control and high-dose groups at the end of the treatment and recovery period; liver and injection sites were also evaluated in low and mid-dose groups at the end of the treatment period.

Dosing solutions initially met acceptance criteria at the high dose ((b) (4) %). The low and mid-dose were (b) (4) % and (b) (4) % of nominal concentration initially. At all doses, the concentrations gradually dropped as the study progressed. At the end of study, dosing solutions were 63.4%, 76.1%, and 84.1% of nominal concentration at 0.007, 0.021, and 0.07 mg free base/mL, respectively. Based on these results, the doses listed above represent the actual concentrations measured.

Main Results:

Clinical signs: The injection sites were swollen in all test article-treated groups from Days 17-20 onward. The severity and incidence were dose dependent (mild at the low and mid dose; mild-severe at the high dose). The swelling regressed during the recovery period.

Gross necropsy: On Day 29, swelling and/or redness was noted in the injection sites in all dose groups, including controls, without a clear dose relationship. The redness and/or swelling persisted in the controls (1 male and 1 female) and high-dose males (both males) through the end of the recovery period.

Histopathology: On day 29, injection site reactions were observed in control and test article-treated groups without a dose relationship. These findings included edema, fibrosis, crust formation, mixed cell infiltration, and/or hemorrhage. In general, the severity ranged from minimal to moderate.

The incidence of injection site edema (moderate) was in overall higher at the high dose, i.e., 1 male control, 3 high-dose males, 1 high-dose female. The incidence of grade 3 or 4 injection site fibrosis (moderate to marked) was also higher at the low-dose or high-dose animals. However, the lack of a dose response suggests the finding

is not test article related. At recovery, only injection site edema (moderate) and hemorrhage (minimal to moderate) were noted in control and high-dose groups.

Except for a higher incidence of edema at the high dose, these findings appear related to the injection procedure rather than related to test article or vehicle. It was stated in the report that these lesions were related to a phlebitis secondary to the treatment methodology, rather than to a direct test article-related effect.

TK: Plasma exposure of AR-13324 free base increased with dose but the increase was greater than dose proportional. Half-life values ranged from ~3-25 hours. Accumulation was observed between Day 1 and Day 7; no further accumulation was observed between Day 7 and Day 28. However, as noted above, the dosing solution concentrations gradually dropped as the study progressed, and this may have had an impact on the ability to assess drug accumulation. No consistent gender-related differences were apparent. The table below shows the mean C_{max} and $AUC_{0\text{-}24hrs}$ values observed on Day 28:

Table 10: TK Parameters: 28-Day GLP Intravenous Toxicity Study in Dogs

Dose (mg/kg/day)*	C_{max} (ng/mL)		$AUC_{0\text{-}24hr}$ (ng•hr/mL)	
	Male	Female	Male	Female
0.07	5.56	4.76	46.28	36.63
0.21	13.1	14.5	54.65	108.61
0.7	63.3	51.5	481.68	334.79

*Nominal dose

(b) (4) was present in the plasma samples of high-dose animals at the 0.167, 0.5, and/or 2 hour timepoints on Days 1, 7, and 28 (C_{max} ≤ 3.38 ng/mL; AUC_{last} ≤ 3.95 ng•hr/mL). No accumulation was apparent with repeated dosing. The study report states that the ratios of (b) (4) AUC_{last} and AR-13324 AUC_{last} (0.0024-0.0095) or (b) (4) C_{max} and AR-13324 C_{max} (0.040-0.059) are consistent with a chiral AR-13324 purity of >94.9%.

AR-13324 free base (b) (4) metabolized by ester hydrolysis to AR-13503 (b) (4) AR-13503 was detected in high-dose animals on Days 7 and Day 28 (not on Day 1) for up to 8 or 14 hours. The C_{max} was ≤2.09 ng/mL; $AUC_{0\text{-}last}$ was 39.57 ng•hr/mL. (b) (4) was not detected.

Other parameters: There were no test article-related effects all other parameters evaluated.

NOAEL: The applicant determined the highest dose to be the NOAEL (i.e., nominally 0.7 mg AR-13324 free base/kg/day; 0.53-0.67 mg/kg/day based on dose formulation analysis). This reviewer concurs.

7 Genetic Toxicology

These studies were previously reviewed during the initial IND.

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Bacterial Reverse Mutation Assay

Study no.:	AR-13324-IS01
Study report location:	EDR Module 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 8, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AR-13324, batch # 0430900,99.4% pure per Certificate of Analysis

Key Study Findings:

- AR-13324 was negative for mutagenicity, under the conditions of this study.
- Toxicity was observed at ≥20 or ≥60 µg/plate for all *Salmonella* strains in the absence of S9 fraction or at ≥200 µg/plate for all other conditions.

Methods:

Strains:	*Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 uvrA
Concentrations in definitive study:	0, 0.20, 0.60, 2.0, 6.0, 20 and 60 µg/plate with all *Salmonella* tester strains in the absence of S9 fraction and 0, 2.0, 6.0, 20, 60, 200 and 600 µg/plate with the remaining test conditions
Basis of concentration selection:	Initial toxicity-mutation test at 1.5-5000 µg/plate ± S9 fraction
Negative control:	Water
Positive control:	With S9 fraction: 2-aminoantracene1.0-15 µg/plate Without S9 fraction: 2-nitrofluorene 1 µg/plate (TA98), sodium azide 1.0 µg/plate (TA100 and TA1535), 9-aminoacridine 75 µg/plate (TA1527), and (b) (4) 1000 µg/plate (WP2 *uvrA*)
Formulation/Vehicle:	Sterile water
Incubation & sampling time:	48-72 hours after overlay had solidified

Study Validity: The study was valid according to regulatory standards.

Results:

Initial Toxicity Mutagenicity Assay: No positive mutagenic responses were observed. However, in the absence of S9 activation, AR-13324 was highly toxic at concentrations ≥15 or ≥50 µg/plate for all *Salmonella* tester strains. In the presence of S9 fraction or for *E. coli* WP2 uvrA, AR-13324 was highly toxic at ≥150 or ≥500 µg/plate. A non-interfering precipitate was observed at ≥500 or ≥1500 µg/plate. A retest was conducted for TA100 and TA1537 (most sensitive strains) at concentrations of 0.2-60 µg/plate in the absence of S9 fraction. Toxicity was observed at ≥20 µg/plate.

Confirmatory Mutagenicity Assay: No positive mutagenic responses were observed. A non-interfering precipitate was observed at 600 µg/plate with all *Salmonella* strains in the presence of S9 activation. Toxicity was observed beginning at 20 or 60 µg/plate for all *Salmonella* strains in the absence of S9 fractions or at 200 µg/plate for all other conditions.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK+/-Mouse Lymphoma Assay

Study no.:	AR-13324-IS02
Study report location:	EDR Module 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 4, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AR-13324, batch # 0430900, 99.4% pure per Certificate of Analysis

Key Study Findings

- AR-13324 was negative for mutagenicity, under the conditions of this study.
- AR-13324 was highly toxic with complete inhibition of suspension growth at ≥5.0 µg/mL.

Methods

Cell line:	L5178Y/TK+/- mouse lymphoma cells
Concentrations in definitive study:	0.1-2 µg/mL (4 hours no S9), 3-7 µg/mL (4 hours + S9), 0.5-1.2 µg/mL (24 hours no S9)
Basis of concentration selection:	Preliminary toxicity assay at 0.5-4540 µg/mL ± S9 fraction
Negative control:	Water
Positive control:	(b) (4) 15 and 20 µg/mL 7,12-Dimethylbenz(a)anthracene 1.5 and 1.25 µg/mL

Formulation/Vehicle: Sterile water
Incubation & sampling time: 4 hours ± S9 faction and 24 hours no S9 fraction

Study Validity: The dosing formulation analysis for the 0.02 and 0.05 mg/mL were 71.4 and 56.0% (backup sample; original sample was 8.3%) of label claim, respectively. The 0.07 mg/mL formulation was initially 74.2% of label claim. The backup sample was 91.3% of label claim. The 0.2 and 2 mg/mL formulations levels were >94.6%. All other criteria for a valid test were met.

Results

Preliminary Toxicity Assay: Suspension growth relative to the solvent controls was 0% at ≥5.0 µg/mL ± S9 fraction with a 4-hour exposure and without activation with a 24-hour exposure. Visible precipitate was present at ≥50 µg/mL. Based on these results, the concentrations treated in the mutagenesis assay ranged from 3 to 7.0 µg/mL in the 4-hour exposure + S9 fraction, 0.1 to 2.0 µg/mL in the 4-hour exposure no S9 fraction, and 0.5-1.2 µg/mL in the 24-hour continuous exposure.

Mutagenicity Assay: There was no concentration-related increase in mutant frequency. No visible precipitate was present at any concentration. There was a dose-dependent decrease in % relative growth. At the highest doses evaluated of 1.5 µg/mL (4 hours no S9), 7 µg/mL (4 hours + S9), and 1.1 µg/mL (24 hours no S9) for mutant frequency, the % relative growth was 14-21%, 19-28%, and 16-18%, respectively.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Micronucleus Test Performed as Part of Study AR-13324-AS03: A 7-Day GLP Intravenous Toxicity Study of AR-13324 in Rats with a 7-Day Recovery

Study no: AR13324-IS03
Study report location: EDR Module 4.2.3.1
Conducting laboratory and location: (b) (4)

Key Study Findings

- AR-13324 did not increase the incidence of micronuclei formation in rat bone marrow, under the conditions of this study.

Methods

Doses in definitive study:	0, 1, 3, and 10 mg/kg (see Study # AR-13324-AS03)
Frequency of dosing:	1x/day for 7 days
Route of administration:	IV bolus injection
Dose volume:	10 mL/kg
Formulation/Vehicle:	Sodium Chloride Injection, 0.9%
Species/Strain:	Bone marrow slides from Sprague-Dawley rats
Number/Sex/Group:	One slides from the first 5 male rats in each group An additional 5 slides were evaluated from the control and high-dose males in the 1-week recovery period.
Satellite groups:	None
Basis of dose selection:	Results of acute IV toxicity Study # AR-13324-AS01 (see above)
Negative control:	Vehicle treated animals
Positive control:	None

Study Validity: The study did not have a positive control. However, according to ICH Guidance S2(R1), it is considered sufficient to treat animals with a positive control only periodically, after a laboratory has established competence in use of the assay. (b) (4) has a well-established reputation for the conduct of genetic toxicity studies. Other aspects of the study followed regulatory standards.

Results: The ratio of polychromatic erythrocytes (PCE)/total erythrocytes was slightly lower at all AR-13324 doses (-5, -7, and -11% at 1, 3, and 10 mg/kg, respectively) suggesting slight toxicity to the bone marrow. There was no increase in the incidence of micronucleated PCE following a 7-day treatment with AR-13324 at doses ≤10 mg/kg/day IV, or following a 7-day recovery period.

8 Carcinogenicity

No studies have been conducted.

The applicant provided a justification requesting a waiver not to conduct the studies. Based on the following observations, the reviewer agrees the existent data support that carcinogenicity evaluation is not relevant for the intended dosing regimen.

- Toxicokinetic results of the various topical ocular safety studies performed in rabbits and monkeys, showed low levels of netarsudil (close to the lower limit of quantitation of <1 ng/mL), its enantiomer, and their metabolites.

- In Phase 1 pharmacokinetic study in healthy volunteers (AR-13324-CS101), there were no observed plasma netarsudil concentrations above the lower limit of quantitation (LLOQ, 0.100 ng/mL) at any timepoint in any subject.
    - According to information in the proposed label, only 1 plasma sample from 1 subject had a concentration above the LLOQ for the primary metabolite of netarsudil (0.11 ng/mL).

- There were no signs of pre-neoplastic lesions following chronic repeated dosing in a 6-month topical ocular toxicity study in rabbits (Study # AR-13324-AS13) and a 9-month topical ocular toxicity study in primates (Study # AR-13324-AS14).

- The ocular tissue distribution studies in Dutch Belted rabbits (Study # AR-13324-APK03) showed slower terminal elimination in retina-choroid-plexus, lens and iris/ciliary body (with $t_{1/2,e}$ values ranging from 68 to 204 hours) after a single topical dose. However, these tissues did not show indications of local tissue reactions or other pathophysiological responses in the long-term ocular toxicity studies.

    - A single IV dose mass balance/autoradiography study in pigmented rats (Study # AR-13324-APK04) showed radioactivity in some tissues persisted throughout the duration of the study (168 hours).
    - These tissues included the adrenal gland cortex, lung, liver, adrenal gland medulla, preputial gland, eye uvea, exorbital lacrimal gland, intra-orbital gland.
    - However, the long-term ocular toxicity studies did not show local tissue reactions or other pathophysiological responses in these tissues.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

No studies were conducted.

9.2 Embryonic Fetal Development

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)

Study no.:	AR-13324-AS21
Study report location:	EDR Module 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 29, 2015
GLP compliance:	Yes, except for dose formulations analysis. The dose formulations analyses were performed under GMP (21 CFR Parts 210 and 211).
QA statement:	Yes
Drug, lot #, and % purity:	AR-13324, batch # 0459933, 97.8-99.2% pure (b) (4) batch # 0450494, 97.8% pure Dosing solutions consist of a combination of both test articles at a ratio of 95:5 of AR-13324 (b) (4)

Key Study Findings

- The administration AR-13324 intravenously through a VAP once daily, from GD 6 through 17, resulted in clinical signs indicative of possible abortions, increased resorptions and reduced fetal viability at 0.3 and 3 mg/kg/day.

- Signs of a possible abortion (red, brown or black vaginal discharge, often accompanied by red staining on the cage tray paper), were recorded for a total of 20 adult females (9 females treated with 0.3 mg/kg/day and 11 treated with 3 mg/kg/day) on GDs 13 through 17.

    - Three females (two at 0.3 mg/kg/day and one Toxicokinetic group female at 3 mg/kg/day) were euthanized during the treatment period because of overall deteriorating health following a possible abortion.

- None of the 24 gravid females treated with 3 mg/kg/day, and 16 of the 24 gravid females treated with 0.3 mg/kg/day had viable fetuses in their litters at C-section.

- There was a slight increase in the mean number of ossified caudal vertebrae at ≥0.1 mg/kg/day, and forelimb phalanges at ≥0.03 mg/kg/day.

- Based on the signs of abortions, increased resorptions and reduced fetal viability at ≥0.3 mg/kg/day, the NOAEL for both maternal and adverse embryofetal effects is considered to be the 0.1 mg/kg/day nominal dose level.

Methods

Nominal Doses:	0 (vehicle control), 0.03, 0.1, 0.3, and 3 mg/kg/day **Note:** Based on dosing solution analysis, the actual values were 0.022 and 0.08 mg/kg/day at 0.03 and 0.1 mg/kg/day, respectively.
Frequency of dosing:	Once daily
Dose volume:	5 mL/kg
Route of administration:	Slow bolus injection via a VAP (jugular vein) **Note:** The use of a central venous catheter (accessed by a VAP) was intended to reduce local irritation previously noted in peripheral venous injection studies.
Formulation/Vehicle:	0.9% Sodium Chloride Injection, USP
Species/Strain:	Hsd:SD Sprague Dawley rats
Number/Sex/Group:	24
Satellite groups:	9 females at each dose (including controls) were assigned to TK groups.
Study design:	AR-13324 was administered intravenously through an indwelling jugular vein catheter, from gestation days (GD) 6 through 17. Females were euthanized on GD 20.
Deviation from study protocol:	None with an impact on the interpretation of the data

Observations and Results

Mortality

Two females at 0.3 mg/kg/day (# 4195 and 4196) were euthanized on GD 15, and one female at 3 mg/kg/day (# 4250) on GD 14, due to deteriorating health and signs of a possible abortion.

Both females at 0.3 mg/kg/day exhibited mild decreased activity, medium to large amount of red discharge at the vaginal opening, a small to large amount of red staining on each animal's cage tray paper, pale eyes and ears, hunched posture, and piloerection on GDs 13/14, prior to euthanasia for humane reasons on GD 15. One of these females was also cool to the touch on GD 14.

The female at 3 mg/kg/day belonged to the TK group. Per protocol, the clinical observations were not recorded or included in the report.

Clinical Signs

A total of 20 dams at doses ≥0.3 mg/kg/day exhibited signs of a possible abortion on GDs 13 through 17, as summarized in Table 11 (excerpted from the study report). It was noted in the study report that red vaginal discharge, which was often accompanied by brown or black vaginal discharge, could be an indicator of a spontaneous abortion. Red discharge on the cage tray paper was also considered indicative of a spontaneous abortion in this study, since it was generally accompanied by either red or brown/black vaginal discharge (except for females # 4181, 4192 and # 4202).

Table 11: Incidence of Possible Abortions - Rat Embryofetal Development Study

Group No.	Dose Level (mg/kg/day)	Animal No.	Gestation Day(s) when Observed	Incidence (%) per Group
4	0.3	4181	RSP: 17	38
		4189	RVD: 14, 15 BVD: 14, 16, 17 RSP: 15, 16, 17	
		4190	RVD: 14, 15	
		4191	RVD: 14, 16, 17 BVD: 15	
		4192	RSP: 14	
		4193	RVD: 14 BVD: 15 RSP: 14	
		4194	RVD: 13, 14, 15 BVD: 15 RSP: 13	
		4195	RVD: 14 RSP: 13, 14	
		4196	RVD: 13, 14	
			RSP: 14	
5	3	4202	RSP: 15, 16	46
		4203	RVD: 17 BVD: 15, 16	
		4204	RVD: 15, 16 RSP 16, 17	
		4206	RVD: 14, 15 RSP: 14, 15	
		4207	RVD: 14, 15 BVD: 15, 16 RSP: 15, 16	
		4208	RVD: 13, 14 BVD: 15 RSP: 15	
		4209	RVD: 14 BVD: 14, 15	
		4210	RVD: 14	
		4212	BVD: 14	
		4215	RVD: 14 BVD: 13	
		4217	RVD: 14 BVD: 14	

RVD = Red Vaginal Discharge
BVD = Brown/Black Vaginal Discharge
RSP = red Staining on Cage Paper

Additional clinical signs included pale ears and eyes. The two females at 0.3 mg/kg/day (# 4195 and # 4196) that were euthanized on GD15 showed more severe clinical signs (decreased activity, hunched posture, piloerection, cool to touch). Animal # 4195 had large amount of vaginal red discharge and animal # 4196 had large amount of red staining of the cage paper on Day 14. The amount of discharge in all other animals was small to medium.

Body Weight

Decreased mean body weight was observed at 0.3 mg/kg/day and 3.0 mg/kg/day from GD 15 onward (statistically significant at the high dose at all timepoints and from GD15-17 at the 0.3 mg/kg/day), compared to control group. On GD 20, the decreased was 6% and 17% ($p \leq 0.05$), respectively. Similarly, mean body weight gain was decreased accounting for a reduction of 13% at 0.3 mg/kg/day and 52% ($p \leq 0.05$) at 3 mg/kg/day during the entire gestation period (GD 0-20).

The uterine weight was decreased at 0.3 mg/kg/day and 3.0 mg/kg/day, i.e., 17% and 95% ($p \leq 0.001$), respectively. When corrected for uterine weight, there was no decrease in body weight at any dose level. Therefore, the decreased body weight/body weight gain appears to be related to embryofetal loss/abortion.

Feed Consumption

The study report indicates there were no effects on food consumption. On GD 15 and GD 17, a slight decrease in mean food consumption was noted at 0.3 mg/kg/day (4-9%) and 3 mg/kg/day (6-7%; $p \leq 0.01$ or ≤ 0.05), compared to control group. However, no difference was noted on GD 20.

Toxicokinetics

The mean TK parameters are summarized in Table 12. AR-13324 was detected at all dose levels. Plasma levels increased with dose, but in a greater than dose-proportional manner. Exposure was comparable after single and multiple dosing. Peak levels occurred at 0.167 hours postdose. Plasma levels were still detected at 24 hours postdose at the high dose, but they were below the lower limit of quantitation (BLQ) (<1.0 ng/mL) at earlier timepoints at lower doses.

(b) (4) plasma levels were BLQ (<1.0 ng/mL) at doses of 0.03 to 0.3 mg/kg/day. At 3.0 mg/kg/day, peak plasma levels ranged from 6.19 to 9.93 ng/mL, with peak levels occurring at 0.167 hours postdose. Plasma levels decreased to BLQ by 4 hours postdose.

Metabolites AR-13503 was observed at ≥0.3 mg/kg/day, whereas metabolite (b) (4) (b) (4) was observed at 3 mg/kg/day. Exposure to AR-13503 was greater after multiple dosing of AR-13324 salt based on a comparison of AR-13503 C_{max} and AUC values on GD 6 and 17. Exposure to the metabolite (b) (4) was similar after single or multiple dosing of AR-13324 salt.

Table 12: Summary of TK Parameters - Rat Embryofetal Development Study

Analyte:	AR-13324								AR-13503							
TK Parameters (units)	Gestational Day 6				Gestational Day 17				Gestational Day 6				Gestational Day 17			
Dose (mg salt/kg/day)	0.03	0.1	0.3	3	0.03	0.1	0.3	3	0.03	0.1	0.3	3	0.03	0.1	0.3	3
C_0 (ng/ml)	1.04	5.07	25.2	226	0.707	5.76	18.1	309	not applicable							
C_{max} (ng/ml)	1.04	3.36	15.1	157	0.707	4.07	12.6	194	a	a	1.81	12.2	a	a	0.927	25.8
T_{max} (hr)	0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.167	-	-	0.167	0.167	-	-	0.5	0.167
C_{last} (ng/ml)	1.04	1.48	1.65	0.907	0.707	2.04	1.87	1.79	a	a	1.81	1.28	a	a	0.373	5.84
T_{last} (hr)	0.167	0.5	4	24	0.167	0.5	4	24	-	-	0.167	24	-	-	2	8
$AUC_{0\text{-last}}$ (hr*ng/ml)	0.1737	1.510	15.21	327.8	0.1181	1.838	16.12	420.0	a	a	0.1511	17.90	a	a	1.354	52.63
$AUC_{0\text{-24hr}}$ (hr*ng/ml)	0.3468	2.620	18.51	327.8	0.2358	3.368	19.86	420.0	a	a	0.4525	17.90	a	a	1.727	99.35
$T_{1/2ez}$ (hr)	a	a	1.34	4.68	a	a	1.52	5.26	a	a	a	9.01	a	a	a	6.71
$AUC_{0\text{-inf}}$ (hr*ng/ml)	a	a	17.42	333.8	a	a	19.35	433.1	a	a	a	34.44	a	a	a	98.82
Cl (l/hr/kg)	a	a	17.2	8.97	a	a	15.5	6.93	not applicable							
Al	-	-	-	-	a	a	1.11	1.30								
Analyte:	AR-13323								AR-13534							
TK Parameters (units)	Gestational Day 6				Gestational Day 17				Gestational Day 6				Gestational Day 17			
Dose (mg salt/kg)	0.03	0.1	0.3	3	0.03	0.1	0.3	3	0.03	0.1	0.3	3	0.03	0.1	0.3	3
C_0 (ng/ml)	a	a	a	11.9	a	a	a	12.9	not applicable							
C_{max} (ng/ml)	a	a	a	7.80	a	a	a	8.11	a	a	a	0.923	a	a	a	2.23
T_{max} (hr)	-	-	-	0.167	-	-	-	0.167	-	-	-	0.167	-	-	-	0.167
C_{last} (ng/ml)	a	a	a	0.750	a	a	a	1.14	a	a	a	0.667	a	a	a	0.450
T_{last} (hr)	-	-	-	2	-	-	-	2	-	-	-	0.5	-	-	-	0.5
$AUC_{0\text{-last}}$ (hr*ng/ml)	a	a	a	5.574	a	a	a	5.815	a	a	a	0.3418	a	a	a	0.6324
$AUC_{0\text{-24hr}}$ (hr*ng/ml)	a	a	a	6.324	a	a	a	6.955	a	a	a	0.8421	a	a	a	0.9699
$T_{1/2ez}$ (hr)	a	a	a	0.582	a	a	a	0.727	a	a	a	a	a	a	a	a
$AUC_{0\text{-inf}}$ (hr*ng/ml)	a	a	a	6.175	a	a	a	6.940	a	a	a	a	a	a	a	a

[a] Could not be calculated from the concentration-time data.

Dosing Solution Analysis

Dosing solutions were 72.7, 76.0 and 84.9% of label claim for the 0.006, 0.02 and 0.06 mg/ml nominal formulation concentrations (first day of use), and 95.3% (first day of use) and 94.4% (last day of use) for the 0.6 mg/ml nominal formulation concentration. The 0.006 and 0.02 mg/ml formulations (i.e., stocks for the 0.03 and 0.1 mg/kg/day dose levels, respectively) failed to meet the (b) (4) % acceptance criteria for nominal total salt concentration.

Chiral impurity concentration ranged from (b) (4) % at the beginning and end of study (analyzed only for the 0.6 mg/mL dosing formulation).

Necropsy

Maternal gross findings included pale liver in 1 dam at 0.1 mg/kg/day (# 4171), all organs pale in color in 2 dams at 0.3 mg/kg/day (# 4195 and 4196 euthanized on GD 15), dark red substance in the stomach in dam # 4195, white blotches in the liver in dam # 4196, black substance in the stomach in 1 dam (# 4207) at 3 mg/kg/day, and pale areas in the kidney in 2 additional dams at 3 mg/kg/day (# 4215 and # 4216).

The study report indicates these gross lesions were possibly indicative of uterine bleeding or spontaneous abortion and the dark red/black substance in the stomach could presumably be dried blood from grooming the fur around the vaginal opening. It is also possible that the finding is related to cannibalization of contents of abortion.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

A test article related effect was observed in the number of viable fetuses, early resorptions and post-implantation loss at ≥0.3 mg/kg/day.

None of the 24 gravid adult females at 3 mg/kg/day, and 16 of the 24 gravid adult females at 0.3 mg/kg/day had viable fetuses, compared to all (24) gravid adult control females with viable fetuses in their litter.

Statistically significant increases in mean number of early resorptions (14.9), percent early resorptions (100%) and % post-implantation loss (100%) were observed at 3 mg/kg/day, compared to the control group. Less pronounced (non-statistically significant) effects in the mean number of early resorptions (5.5), percent early resorptions (38%) and % post implantation loss (38%) were observed at 0.3 mg/kg/day, compared to control values (control values: early resorptions = 1.0, percent early resorptions = 8%, percent post-implantation loss = 9%).

The litter (Table 13) and fetal (Table 14) incidence for these findings are shown below:

Table 13: Litter Incidence of C-Section Findings – Rat Embryofetal Development Study

Group #		Early Resorptions			Late Resorptions			Viable Fetuses			Non-Viable Fetuses		
		Left	Right	Total	Left	Right	Total	Left	Right	Total	Left	Right	Total
1	(n)	24	24	24	24	24	24	24	24	24	24	24	24
	Total	9	9	14	0	1	1	24	24	24	0	0	0
	Litter Incidence (%)	37.5	37.5	58.3	0.0	4.2	4.2	100.0	100.0	100.0	0.0	0.0	0.0
2	(n)	24	24	24	24	24	24	24	24	24	24	24	24
	Total	10	9	13	0	0	0	24	23	24	1	1	2
	Litter Incidence (%)	41.7	37.5	54.2	0.0	0.0	0.0	100.0	95.8	100.0	4.2	4.2	8.3
3	(n)	23	23	23	23	23	23	23	23	23	23	23	23
	Total	9	8	15	0	0	0	23	22	23	1	0	1
	Litter Incidence (%)	39.1	34.8	65.2	0.0	0.0	0.0	100.0	95.7	100.0	4.3	0.0	4.3
4	(n)	24	24	24	24	24	24	24	24	24	24	24	24
	Total	12	16	17	0	0	0	16	16	16	0	0	0
	Litter Incidence (%)	50.0	66.7	70.8	0.0	0.0	0.0	66.7	66.7	66.7	0.0	0.0	0.0
5	(n)	24	24	24	24	24	24	24	24	24	24	24	24
	Total	24	23	24	0	0	0	0	0	0	0	0	0
	Litter Incidence (%)	100.0#U	95.8#U	100.0#U	0.0	0.0	0.0	0.0#K	0.0#K	0.0#K	0.0	0.0	0.0

#U = Mann-Whitney U Test at 0.001 level. #K= Kruskal-Wallis Test at 0.001 level.

Group 1: 0 (vehicle control); Group 2: 0.03 mg/kg/day; Group 3: 0.1 mg/kg/day; Group 4: 0.3 mg/kg/day; Group 4: 3 mg/kg/day

Table 14: Fetal Incidence of C-Section Findings – Rat Embryofetal Development Study

Group #		Total Fetuses Male	Female	All	Male Sex Ratio	Means Fetal Weight (g)	Means Uterus Weight (g)	Resorptions % Early	% Late	% Non-Viable	Implantation Loss % Pre-	% Post
1	(n)	24	24	24	24	24	24	24	24	24	24	24
	Total	160	131	291	13.08	90.6	1669	196.8	8.3	0.0	399.5	205.2
	Mean	6.7	5.5	12.1	0.545	3.78	69.5	8.20	0.35	0.00	16.64	8.55
	SDevs	2.41	1.98	2.79	0.1521	0.191	13.61	9.145	1.701	0.000	14.995	9.307
2	(n)	24	24	24	24	24	24	24	24	24	24	24
	Total	182	166	348	12.59	91.0	1927	201.9	0.0	11.6	247.7	213.3
	Mean	7.6	6.9	14.5 +U	0.525	3.79	80.3 *U	8.41	0.00	0.48	10.32	8.89
	SDevs	2.22	2.32	2.81	0.1178	0.290	11.72	11.069	0.000	1.636	11.329	10.917
3	(n)	23	23	23	23	23	23	23	23	23	23	23
	Total	149	161	310	10.86	89.8	1776	185.1	0.0	6.7	270.7	191.2
	Mean	6.5	7.0	13.5	0.472	3.90 +U	77.2	8.05	0.00	0.29	11.77	8.31
	SDevs	2.39	2.04	2.73	0.1482	0.210	13.93	9.248	0.000	1.390	10.606	9.310
4	(n)	24	24	24	16	16	22	24	24	24	24	24
	Total	112	99	211	8.48	62.9	1266	921.6	0.0	0.0	307.8	921.6
	Mean	4.7	4.1	8.8	0.530	3.93 #U	57.5	38.40	0.00	0.00	12.83	38.40
	SDevs	3.71	3.31	6.46	0.1258	0.239	33.52	44.961	0.000	0.000	13.126	44.961
5	(n)	24	24	24	-	-	24	24	24	24	24	24
	Total	0	0	0	-	-	86	2400.0	0.0	0.0	270.5	2400.0
	Mean	0.0 #K	0.0 #K	0.0 #K	-	-	3.6 #K	100.00 #K	0.00	0.00	11.27	100.00 #K
	SDevs	0.00	0.00	0.00	-	-	1.67	0.000	0.000	0.000	11.509	0.000

(-) – Data unavailable
#K= Kruskal-Wallis Test Significant at 0.001 level.
*U= Mann-Whitney U Test Significant at 0.05 level. +U= Mann-Whitney U Test Significant at 0.01 level.
#U= Mann-Whitney U Test Significant at 0.001 level.

Group 1: 0 (vehicle control); Group 2: 0.03 mg/kg/day; Group 3: 0.1 mg/kg/day; Group 4: 0.3 mg/kg/day; Group 4: 3 mg/kg/day

Offspring (Malformations, Variations, etc.)

There was a slight increase in the mean number of ossified caudal vertebrae at ≥0.1 mg/kg/day and forelimb phalanges at ≥0.03 mg/kg/day (Table 15).

Table 15: Fetal Ossification Findings - Rat Embryofetal Development Study

GROUP		1	2	3	4	5
TREATMENT		VEHICLE CONTROL	AR-13324	AR-13324	AR-13324	AR-13324
DOSE LEVEL (MG/KG/DAY)a		0	0.03	0.1	0.3	3
LITTERS EXAMINED	N	24	24	23	16	0
FETUSES EXAMINED	N	151	178	160	111	0
OSSIFICATION SITES PER FETUS PER LITTER						
HYOID	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	
VERTEBRAE						
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	
THORACIC	MEAN±S.D.	13.13 ± 0.23	13.13 ± 0.17	13.11 ± 0.16	13.11 ± 0.12	
LUMBAR	MEAN±S.D.	5.86 ± 0.23	5.86 ± 0.17	5.88 ± 0.16	5.86 ± 0.13	
SACRAL	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	
CAUDAL	MEAN±S.D.	4.15 ± 0.25	4.28 ± 0.32	4.38 ± 0.31*	4.46 ± 0.35**	
RIBS (PAIRS)	MEAN±S.D.	13.10 ± 0.18	13.10 ± 0.12	13.08 ± 0.14	13.09 ± 0.10	
FORELIMB b						
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
METACARPALS	MEAN±S.D.	3.99 ± 0.04	3.99 ± 0.04	4.00 ± 0.00	4.00 ± 0.00	
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	
PHALANGES	MEAN±S.D.	6.72 ± 0.27	7.03 ± 0.30*	7.12 ± 0.42**	7.16 ± 0.55**	

Similar effects on early resorption and fetal viability were noted in the dose-range finding study at doses of 0.3-10 mg/kg, as noted below.

A Dose-Range-Finding Study for Effects of AR-13324 Following Intravenous Administration via an Indwelling Jugular Vein Catheter on Embryo-Fetal Development in Rats (SEG II) (Study # AR-13324-AS18; non-GLP; Module 4.2.3.5.2) - Sprague Dawley female rats (5 in control group; 7 in test-article treated groups) were administered AR-13324/ (b) (4) (95%:5% (w/w)) intravenously through an indwelling jugular vein catheter, from GD 6 through 17, at nominal dose levels of 0, 0.3, 3 and 10 mg/kg/day (actual dose levels of 0, 0.09, 2.4 and 10.0 mg/kg/day, respectively). Females were euthanized on GD 20. Each fetus was weighed, examined macroscopically and subsequently euthanized.

The main findings included:

- Clinical signs indicative of a spontaneous abortion (presence of red vaginal discharge) were observed beginning on GD 13 or 14 in 1, 3, and 7 females at 0.3, 3 and 10 mg/kg/day, respectively. Of these, 1, 1 and 5 females at 0.3, 3 and 10 mg/kg/day, respectively, were euthanized prior to the scheduled Cesarean section on GD 20 (i.e., GD13, 14, 15 or 20).
    - Two of the euthanized high-dose females had moderate or severe red vaginal discharge. All euthanized females showed clinical signs of pale extremities, generalized paleness, decreased activity, red urogenital staining, red staining of the snares, and/or red staining of the cage paper or cage wire floor.
    - Gross lesions at necropsy included the presence of a red vaginal substance at the vaginal opening/in the vagina/at the site of the cervix, the presence

of a black substance or red substance in the stomach, and/or enlarged spleen.

- Statistically significant daily mean weight losses (68% during GD 6-17 interval) and reduced food consumption (≤32%) were recorded generally beginning on GD 13 for females treated with 10 mg/kg/day. Food consumption was also decreased (≤12%) at 3 mg/kg/day starting on GD 15 (statistical significance only on GD 15).
    - The mean uterine weight of females at 10 mg/kg/day (0.9 gram) was markedly lower than the mean uterine weight of vehicle control females (as expected, given the 100% early resorptions occurring at this dose).
    - The mean corrected (for uterine weight) body weight and corrected body weight change were also lower (5% and 24%, respectively) compared to control group. However, individual animal listings showed corrected body weight values within those observed in control group. It is possible the slight decrease in the mean value reflects the low animal number per group (5 in controls vs 2 at 10 mg/kg/day).

- The number of early resorptions (and % early resorptions) increased with increasing dose, i.e., 0, 1, 3 and 13 (3.2, 19.1, 24.4, and 100%) for the vehicle control, 0.3, 3 and 10 mg/kg/day dose groups, respectively.

- The mean % viable fetuses was decreased (96.8, 80.9, 61.3 and 0%) and the mean % post-implantation loss was increased (3.2, 19.1, 38.7, and 100%) with increasing dose (0, 0.3, 3, and 10 mg/kg/day, respectively), compared to controls.

- There were no fetal external anomalies observed on this study. All viable fetuses appeared normal in the vehicle control group and in females treated with 0.3 or 3 mg/kg/day. There were no viable fetuses from females treated with 10 mg/kg/day.

Therefore, the intended 3 mg/kg/day dose was recommended as the high dose for a subsequent study of embryo-fetal development in rats.

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) (Final Report)

Study no.:	AR-13324-AS22
Study report location:	EDR Module 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 25, 2015
GLP compliance:	Yes, except for dose formulations analysis. The dose formulations analyses were performed under GMP (21 CFR Parts 210 and 211).
QA statement:	Yes
Drug, lot #, and % purity:	AR-13324, batch # 0459933, 97.8-99.2% pure (b) (4) batches # 0450494, 0450494-34, 0450494-52, 97.8-97.9% pure

Notes:

- Dosing solutions consist of a combination of both test articles at a ratio of 95:5 of AR-13324 (b) (4)
- The % purity listed is based on chemical purity (HPLC) for AR-13324 and chiral purity for (b) (4)
- Only data for (b) (4), batch # 0459933 was included, but the study report lists 3 batches (they may be different codes for the same batch)

Key Study Findings

- The % viable fetuses was slightly reduced, % post-implantation loss was slightly increased, and early resorptions (fetal and litter incidences) were increased at the high dose (5.0 mg/kg/day). Although not statistically significant, these slight changes were considered to be test article-related.

- A slight test article related statistically significant decrease in mean fetal weights was observed in litters at 5.0 mg/kg/day.

- The study report states that there were no AR-13324-related fetal external, soft tissue, or skeletal fetal malformations or variations at any dose. However, there were some findings with higher incidence in test-article treated groups including absent intermediate lung lobe (3 fetuses in 2 litters at 5.0 mg/kg/day) and abdominal wall defects (one fetus with an umbilical hernia at 5 mg/kg/day and a

second fetus with thoracogastroschisis at 3.0 mg/kg/day). Manubrium and sternal centra skeletal defects were observed in the fetus with thoracogastroschisis.

- The applicant considered the no observed effect level (NOEL) was 3.0 mg/kg/day. Based on the abdominal wall defects observed at ≥3 mg/kg/day, this reviewer considers the NOEL to be 0.5 mg/kg/day.

Methods

Doses:	0 (vehicle control), 0.5, 3.0, and 5.0 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	1 mL/kg
Route of administration:	Slow bolus injection via a VAP (jugular vein) **Note:** The use of a central venous catheter (accessed by a VAP) was intended to reduce local irritation previously noted in peripheral venous injection studies.
Formulation/Vehicle:	0.9% Sodium Chloride Injection, USP
Species/Strain:	New Zealand White rabbits
Number/Sex/Group:	19
Satellite groups:	Three females at each test article dose level were assigned to TK groups.
Study design:	AR-13324 was administered intravenously from GD 6 through GD 19 via a VAP located over the interscapular region and attached to an indwelling catheter located in the right jugular vein. Females were euthanized on GD 29.
Deviation from study protocol:	None with an impact on the interpretation of the data

Observations and Results

Mortality

High-dose animal #1393 was found dead on GD 9 during the 1-2 hours post-dose observations. Mid-dose animal #1359 was flailing around in the cage and found dead on Gestation Day 13 minutes after dose administration. At necropsy, animal # 1359 had a 4x2 cm mass near the emptying site of the vascular access port. It was stated in the study report that the cause of both deaths is believed to be secondary to the dosing procedure/problems with the catheter, and not test article related.

Clinical Signs

None considered test article related

Body Weight

There were no statistically significant differences in mean body weight or uterine weight, although mean uterine weight was slightly lower (16%) at the high dose. Mean body weights (GD 29) in test article-treated groups were all comparable to control group when corrected for uterine weight.

Non-statistically significant slightly lower mean weight gains of 30%, 50%, and 25% were noted in the high-dose group during the treatment period (GD 6-19), post-treatment period (GD 19-29), and the overall gestation period (GD 0-29), respectively. The mean corrected body weight changes (the GD 0 body weight subtracted from the corrected body weight) were not affected for any of the dose groups. Therefore, the decreased body weight/body weight gain does not appear to be related to maternal toxicity, but to fetal toxicity (reduced fetal weight).

Feed Consumption

No test article related effects

Toxicokinetics

The mean TK parameters are summarized in Table 16. AR-13324 was detected at all dose levels. Plasma levels increased with dose, with dose proportionality between 0.5 and 3 mg salt/kg/day and less than dose-proportionality between 3 and 5 mg kg/day. Exposure was comparable after single and multiple dosing. Peak levels occurred at ~10 min postdose. Depending on the dose, T_{max} occurred between 2-8 hours postdose.

Exposure to (b) (4) was observed at all dose groups and was dose dependent but not dose proportional. No accumulation occurred with repeated dosing. Depending on the dose, T_{max} occurred between 10 minutes to 2 hours postdose.

Metabolite AR-13503 was detected at all doses, whereas metabolite (b) (4) was observed at ≥3.0 mg/kg/day. Metabolite AR-13503 was observed at levels ≤2-fold lower (based on $AUC_{0\text{-last}}$) than parent AR-13324, with T_{max} occurring at 10 minutes to 8 hours postdose. No accumulation of either metabolite occurred with repeated dosing.

Table 16: Summary of TK Parameters – Rabbit Embryofetal Development Study

Analyte:	AR-13324						AR-13503					
TK Parameters (units)	Gestational Day 6			Gestational Day 19			Gestational Day 6			Gestational Day 19		
Dose (mg salt/kg/day)	0.5	3	5	0.5	3	5	0.5	3	5	0.5	3	5
C_0 (ng/ml)	38.6	158	232	33.0	205	233	not applicable					
C_{max} (ng/ml)	26.1	73.7	150	21.4	133	148	7.11	56.2	90.1	8.79	47.5	128
T_{max} (hr)	0.167	0.72	0.28	0.167	0.167	0.167	0.167	0.167	0.28	0.167	0.167	0.167
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
T_{last} (hr)	4	8	8	3.3	8	8	2.7	6.7	8	2.1	8	8
$AUC_{0\text{-last}}$ (hr*ng/ml)	23.6	172	210	20.6	149	163	10.5[b]	77.0	179	6.37	52.2	140
$AUC_{0\text{-24hr}}$ (hr*ng/ml)	27.6	195	247	23.4	165	180	9.39	87.9	229	7.97	65.4	165
$T_{1/2ez}$ (hr)	2.1	2.4	4.3	1.3	1.9	1.9	6.6[b]	1.9	2.8	2.4[b]	4.7	2.6
$AUC_{0\text{-inf}}$ (hr*ng/ml)	29.1	184	236	23.6	155	169	26.4[b]	82.0	205	14.4[b]	63.6	151
Cl (l/hr/kg)	17.6	19.5	22.6	21.6	20.6	29.9	not applicable					
AI	-	-	-	0.81	0.93	0.76						

[a] Could not be calculated from the concentration-time data.
[b] Mean of two; all other values are a mean of three.

Analyte:	AR-13323						AR-13534					
TK Parameters (units)	Gestational Day 6			Gestational Day 19			Gestational Day 6			Gestational Day 19		
Dose (mg salt/kg)	0.5	3	5	0.5	3	5	0.5	3	5	0.5	3	5
C_0 (ng/ml)	1.35	12.9	13.8	1.24[c]	10.3	11.8	not applicable					
C_{max} (ng/ml)	1.35	7.94	8.35	1.24[c]	6.25	6.91	[a]	1.62	3.95	[a]	1.35[b]	3.04
T_{max} (hr)	0.167	0.167	0.28	0.167[c]	0.167	0.167	-	0.167	0.28	-	0.167[b]	0.167
C_{last} (ng/ml)	1.35	1.21	2.25	1.24[c]	1.09	1.60	[a]	1.62	2.25	[a]	1.35[b]	2.09
T_{last} (hr)	0.167	2	1.3	0.167[c]	1.3	1	-	0.167	1.1	-	0.167[b]	0.28
$AUC_{0\text{-last}}$ (hr*ng/ml)	[a]	6.58	6.54	[a]	4.28	4.11	[a]	0.136	1.88	[a]	0.113[b]	0.556
$AUC_{0\text{-24hr}}$ (hr*ng/ml)	0.449	7.79	9.10	0.414[c]	5.01	4.91	[a]	0.406	2.89	[a]	0.338[b]	0.939
$T_{1/2ez}$ (hr)	[a]	1.3	0.73	[a]	0.57	0.42	[a]	[a]	2.4[b]	[a]	[a]	0.20[c]
$AUC_{0\text{-inf}}$ (hr*ng/ml)	[a]	8.71	8.62	[a]	5.13	4.97	[a]	[a]	7.79[b]	[a]	[a]	1.61[c]

[a] Could not be calculated from the concentration-time data.
[b] Mean of two
[c] Mean of one

Dosing Solution Analysis

AR-13324 label claim for the 0.5, 3.0 and 5.0 mg/mL formulations was 96.7 to 99.3%, with the exception of one set of 3.0 mg/mL samples collected approximately halfway through the dosing period which averaged 139.8%.

Chiral impurity concentration ranged from [redacted] (b) (4) % at the beginning and end of study (analyzed only for the 0.6 mg/mL dosing formulation).

Necropsy

At the high-dose, animal #1386 had no left uterine horn, animal #1392 had a 30x20x10 mm mass proximal to the thymus and the subcutaneous surface revealed tan caseous material, and animal #1395 had a constricted right uterine horn. These findings are unlikely test article related.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

There were 0, 2, 3, and 1 females with non-viable fetuses at 0, 0.5, 3.0, and 5.0 mg/kg/day, respectively, at C-section performed on GD 29 (Table 17). The % viable fetuses was slightly reduced and % post-implantation loss was slightly increased at 5.0 mg/kg/day. The increase post-implantation loss was due to an increase in early resorptions (fetal and litter incidences). Although not statistically significant, these slight changes were considered to be test article related. The litter incidence and fetal incidence for these findings are shown in Tables 17 and 18, respectively.

Table 17: Cesarean Section Findings in Rabbits – Litter Incidence on GD 29

		Early Resorptions			Late Resorptions			Viable Fetuses			Non-Viable Fetuses		
Group		Left	Right	Total	Left	Right	Total	Left	Right	Total	Left	Right	Total
1	(n)	14	14	14	14	14	14	14	14	14	14	14	14
	Total	1	0	1	0	0	0	14	14	14	0	0	0
	Litter Incidence (%)	7.1	0.0	7.1	0.0	0.0	0.0	100.0	100.0	100.0	0.0	0.0	0.0
2	(n)	16	16	16	16	16	16	16	16	16	16	16	16
	Total	1	0	1	1	1	2	16	16	16	2	0	2
	Litter Incidence (%)	6.3	0.0	6.3	6.3	6.3	12.5	100.0	100.0	100.0	12.5	0.0	12.5
3	(n)	16	16	16	16	16	16	16	16	16	16	16	16
	Total	2	1	3	0	1	1	16	16	16	1	2	3
	Litter Incidence (%)	12.5	6.3	18.8	0.0	6.3	6.3	100.0	100.0	100.0	6.3	12.5	18.8
4	(n)	14	14	14	14	14	14	14	14	14	14	14	14
	Total	5	1	6	0	0	0	13	11	13	0	1	1
	Litter Incidence (%)	35.7	7.1	42.9	0.0	0.0	0.0	92.9	78.6	92.9	0.0	7.1	7.1

Table 18: Cesarean Section Findings in Rabbits – Fetal Incidence on GD 29

		Resorptions		Fetuses		Implantation Loss	
Dam	Status	% Early	% Late	% Viable	%Dead	% Pre-	% Post-
1	(n)	14	14	14	14	14	14
	Means	1.2	0.0	98.8	0.0	13.0	1.2
	Sdevs	4.45	0.00	4.45	0.00	14.81	4.45
2	(n)	16	16	16	16	16	16
	Means	0.9	1.5	96.4	1.3	6.0	3.6
	Sdevs	3.57	4.04	5.65	3.47	6.73	5.65
3	(n)	16	16	16	16	16	16
	Means	2.4	0.7	94.5	2.4	4.5	5.5
	Sdevs	5.26	2.78	9.17	5.26	7.27	9.17
4	(n)	14	14	14	14	14	14
	Means	11.9	0.0	87.3	0.8	14.1	12.7
	Sdevs	26.18	0.00	25.96	2.97	18.78	25.96

Offspring (Malformations, Variations, etc.)

Fetal evaluations were based on 104, 138, 122, and 94 live GD 29 Caesarean-delivered fetuses in 14, 16, 16, and 13 litters in the 0, 0.5, 3.0, and 5.0 mg/kg/day dose groups, respectively.

A slight decrease (16%; p≤0.01) in mean fetal weights was observed at 5.0 mg/kg/day. The study report indicates that the decrease in fetal body weight likely resulted in the non-statistically significant lower mean weight gain noted in the high-dose pregnant females.

The study report states that there were no AR-13324-related fetal external, soft tissue, or skeletal fetal malformations or variations at any dose. However, there were some findings with higher incidence in test-article treated groups (Tables 19 and 20):

Table 19: Gross External Examinations – Rabbit Study

GROUP		1	2	3	4
TREATMENT		VEHICLE CONTROL	AR-13324	AR-13324	AR-13324
DOSE LEVEL (MG/KG/DAY)a		0	0.5	3.0	5.0
LITTERS EVALUATED	N	14	16	16	13
LITTERS WITH LIVE FETUS(ES)	N	14	16	16	13
FETUSES EVALUATED	N	104	140	125	94b
LIVE	N	104	138	122	93b
DEAD c	N	0	2	3	1
SKIN: DISCOLORATION					
LITTER INCIDENCE	N(%)	1(7.1)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(1.0)	0(0.0)	0(0.0)	0(0.0)
BODY: UMBILICAL HERNIA					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(7.7)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(1.1)
BODY: THORACOGASTROSCHISIS					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(6.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)	0(0.0)

The fetal and litter incidence of umbilical hernia and thoracogastroschisis were higher than historical control values (Charles River Historical Control database, June 2008-2010; see below). As these are rare findings (1 in 17,000 for thoracoschisis; combined incidence of 1 in 1,400 for low-grade umbilical hernia to complete gastroschisis or even thoracogastrosis)[2], a test article-related effect in abdominal wall defects cannot be ruled out.

Historical control incidence:

- Umbilical hernia: Fetal incidence – 0-0.7%; Litter incidence: 0-5.6%
- Thoracogastroschisis: Fetal incidence – 0-0.5%; Litter incidence: 0-4.2%

[2] Palmer AK, Spontaneous malformations of the New Zealand White rabbit: The background to safety evaluation tests, *Lab Anim*, 2:195-206 (1968).

Table 20: Fetal Visceral Examinations – Rabbit Study

GROUP		1	2	3	4
TREATMENT		VEHICLE CONTROL	AR-13324	AR-13324	AR-13324
DOSE LEVEL (MG/KG/DAY)a		0	0.5	3.0	5.0
LITTERS EVALUATED	N	14	16	16	13
LITTERS WITH LIVE FETUS(ES)	N	14	16	16	13
FETUSES EVALUATED	N	104	138	122	94
LIVE	N	104	138	122	94
LUNGS: INTERMEDIATE LOBE ABSENT					
LITTER INCIDENCE	N(%)	1(7.1)	0(0.0)	0(0.0)	2(15.4)
FETAL INCIDENCE	N(%)	1(1.0)	0(0.0)	0(0.0)	3(3.2)**d
STOMACH: PROTRUDES THROUGH THORACIC AND ABDOMINAL OPENING					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(6.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)c	0(0.0)
INTESTINES: PROTRUDES THROUGH THORACIC AND ABDOMINAL OPENING					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(6.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)c	0(0.0)
INTESTINES: PROTRUDES THROUGH UMBILICAL OPENING					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(7.7)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(1.1)d
SPLEEN: PROTRUDES THROUGH THORACIC AND ABDOMINAL OPENING					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(6.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)c	0(0.0)

Note: Not all organs protruding through the thoracic and abdominal cavity in the mid-dose fetus are shown in the table.

Soft tissue examination confirmed the abdominal wall defects noted in one fetus (# 1369-7) at 3.0 mg/kg/day (thoracogastroschisis at external examination) and one fetus (# 1385-6) at 5.0 mg/kg/day (umbilical hernia at external examination). Fetus 1369-7 (3.0 mg/kg/day) had protrusion of various viscera (heart, liver, stomach, intestines, and spleen) through an opening in the thoracic and abdominal walls. Similarly, the intestines of fetus 1385-6 (5.0 mg/kg/day) protruded through an umbilical opening, and the intermediate lung lobe was absent in this fetus.

The number of fetuses with an absent intermediate lung lobe was significantly increased ($p \leq 0.01$) at 5.0 mg/kg/day group compared to controls (fetuses # 1385-6 and -7 and 1390-5 at 5.0 mg/kg/day and fetus 1329-8 in control). The fetal and litter incidence at 5.0 mg/kg/day is higher than that in the Charles River Historical Control database (June 2008-2010) (see below).

Historical control incidence:

- Lungs (one or more lobes agenesis): Fetal incidence – 0-2.2%; Litter incidence: 0-11.8%

A cursory review of the literature revealed existing evidence supporting a role for Rho/Rho-kinase signaling pathway in branching morphogenesis in the fetal mouse and rat lung[3,4] Therefore, a test article related effect cannot be ruled out.

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[3] McMurtry IF et al., Hypoxia and Rho/Rho-kinase signaling: Lung development versus hypoxic pulmonary hypertension, *Adv Exp Med Biol*, 543: 127-37 (2003).

[4] Moore KS et al., Control of basement membrane remodeling and epithelial branching morphogenesis in embryonic lung by Rho and cytoskeletal tension, *Dev Dyn*, Feb; 232(2): 268-81 (2005).

Fetal Skeletal Examinations:

Skeletal examination confirmed the abdominal wall defects noted in fetus 1369-7 in the 3.0 mg/kg/day dose group (thoracogastroschisis at external examination and protruding viscera at soft tissue examination) which was evident as a duplicated manubrium and sternal centra. This fetus also had an incompletely ossified manubrium, incompletely ossified sternal centra, and incompletely ossified pubes.

Two rabbit dose-finding (non-GLP) studies were conducted. Slight adverse fetal findings were observed at 3 mg/kg/day (not observed in the GLP study).

A Dose-Range-Finding Study for Effects of AR-13324 Following Intravenous Administration via a Vascular Access Port on Embryo-Fetal Development in Rabbits (SEG II) **(Study # AR-13324-AS19; non-GLP; Module 4.2.3.5.2) –** Presumed pregnant females NZW rabbits (7/group) were administered IV doses of 0.0.1, 0.3, 1 and 3 mg/kg/day from GD 6 to GD 19, as a slow bolus infusion via a VAP. Females were euthanized on GD 29. Fetuses were weighed, examined macroscopically and subsequently euthanized.

No apparent maternal toxicity was observed. Subtle effects (not statistically significant) on fetal viability at the high dose of 3 mg/kg/day were observed. These included: increased non-viable fetuses, increased % post-implantation loss, and decreased fetal body weight, as detailed below:

- The total number of nonviable fetuses (7 nonviable fetuses) at 3 mg/kg/day was slightly higher than the total number of nonviable fetuses in control group (3 nonviable fetuses).
- The mean percentage of viable fetuses at 3 mg/kg/day was lower (86%), compared to the percentage of viable fetuses in control group (94%).
- The mean percentage of nonviable fetuses (and post-implantation loss) at 3 mg/kg/day was higher (14%) compared to the percentage nonviable fetuses calculated in control group (6%).
- The mean fetal weight was slightly lower (17%; $p \leq 0.05$) compared to the mean fetal weight for control group.

A Dose-Range-Finding Study for Effects of AR-13324 Following Intravenous Administration via a Vascular Access Port on Embryo-Fetal Development in Rabbits (SEG II) **(Study # AR-13324-AS20; non-GLP; Module 4.2.3.5.2) –** The study explored the potential adverse effects at two higher doses (5 and 9 mg/kg/day), in an attempt to produce minimal toxicity in the adult females and some adverse effects on the litters obtained from these adult females. Presumed pregnant NZW female rabbits (7/group) were dosed intravenously, once daily from GD 6 to GD 19, as a slow bolus infusion via a VAP. Females were euthanized on GD 29. Fetuses were weighed, examined macroscopically and subsequently euthanized. There was no concurrent control group in this study; the applicant compared the results to values normally reported for NZW rabbits.

A confirmed abortion and a probable abortion occurred on GD 20 for two females treated with 5 mg/kg/day. The abortion status was probable but not confirmed in one female, since placentae were not observed prior to necropsy. Black fluid at the cervix (possible dried blood), a clear cyst on the left ovary, and hemorrhagic left and right corpora lutea were noted in this female.

On GD 29, main C-section findings included:

- The number of litters with one or more viable fetuses was reduced for both dose groups (67% and 33% of litters for 5 and 9 mg/kg/day, respectively, compared to 100% of litters with viable fetuses normally encountered for healthy NZW females).
- The number of early resorptions (and % early resorptions) per litter were elevated (13% and 10% early resorptions for 5 and 9 mg/kg/day, respectively, compared to less than 10% normally found in litters from healthy NZW females).
- The % viable fetuses were reduced for both groups (54 and 24% viable fetuses, for 5 and 9 mg/kg/day, respectively, compared to 95 to 100% viable fetuses normally expected from healthy NZW females).
- The % post-implantation loss was elevated (13 and 76% implantation losses for the 5 and 9 mg/kg/day, respectively, compared to less than 8% postimplantation loss in healthy NZW females).

Based on the findings of both non-GLP dose-range-finding studies, the 5 mg/kg/day dose level was used for the high dose in the definitive rabbit embryo-fetal development study.

9.3 Prenatal and Postnatal Development

No studies were conducted.

10 Special Toxicology Studies

In the Phase 3 clinical studies, corneal deposits localized to the basal corneal epithelium were observed, which were commonly described by investigators as “corneal whorls” or “corneal verticillata.” AR-13324 is a cationic amphiphilic drug (CAD) that, like other drugs known to induce phospholipidosis (PLD), contains a hydrophobic ring structure and a hydrophilic side chain with a charged amine group. To determine whether the AR-13324-associated corneal deposits observed in humans are a result of PLD, the applicant is investigated the ability of AR-13324 to induce lysosomal accumulation of phospholipids in tissue culture cells.

Evaluation of the Potential of AR-13324 to Induce Phospholipidosis in a Cell-Based Assay (Study # AR-13324-IPH07) – This study assessed the potential of netarsudil mesylate (AR-13324), a related Rho kinase inhibitor (AR-12286), and the active metabolite of AR-13324 (AR-13503) to induce PLD in Chinese hamster ovary (CHO-K1) cells. A fluorescence-based assay was used to visualize intracellular accumulation of phospholipids in control and drug-treated CHO-K1 cells.

AR-13324 induced focal phospholipid accumulation that colocalized with lysosomes at micromolar concentrations (EC_{50} = 1.1 µM; 498.88 ng/mL). Transmission electron microscopy revealed that AR-13324 induced the formation of lamellar bodies in CHO-K1 cells, confirming that the fluorescent phospholipid accumulation in AR-13324-treated cells was due to PLD. PLD was not induced by the related Rho kinase inhibitor (AR-12286) or AR-13324 active metabolite (AR-13503) at concentrations up to 3.3 µM (estimated to be equivalent to 1000 ng/mL, based on a molecular weight of 306 g/mol).

As noted in the study report, these results support the conclusion that, like other cationic amphiphilic drugs, AR-13324 can induce PLD, and that the induction of PLD is not a class effect of Rho kinase inhibitors.

11 Integrated Summary and Safety Evaluation

Netarsudil mesylate is a new chemical entity Rho-associated protein kinase inhibitor (ROCK) being developed as a topical formulation (RHOPRESSA, Netarsudil Ophthalmic Solution 0.02%) for the indication of reduction of intraocular pressure (IOP).

Netarsudil appears to reduce IOP through multiple mechanisms of action. A study in monkeys showed that Netarsudil Ophthalmic Solution lowers IOP by increasing aqueous humor outflow through the trabecular meshwork and decreasing the production of aqueous humor. Studies in rabbits showed that Netarsudil Ophthalmic Solution can reduce episcleral venous pressure (EVP). A study using enucleated human eyes demonstrated that AR-13503 (active metabolite, see below) increases outflow through the human trabecular outflow pathway. This activity was correlated with morphological changes in the trabecular outflow tissues, including an increase in the area of actively filtering trabecular meshwork tissue, expansion of the trabecular meshwork tissue, and dilation of episcleral veins. These studies support (b) (4) section 12.1 Mechanism of Action of the proposed label: increasing trabecular outflow facility, (b) (4)

In vitro studies showed that netarsudil is a potent Rho-kinase inhibitor (K_i = 1.1 to 1.2 nM [~0.5 ng/mL] against both ROCK1 and ROCK2. Netarsudil is metabolized by corneal esterases, and the esterase metabolite of netarsudil, AR-13503, is approximately 5 times more potent as an inhibitor of Rho kinase than the parent compound. AR-13503 was the predominant species found in the aqueous humor after topical ocular dosing in rabbits.

In vitro studies also showed netarsudil has inhibitory activity against 25 additional kinases, besides ROCK1 and ROCK2, and more than 20 non-kinase proteins. The concentrations tested were 0.5 µM (227 ng/mL) or 10 µM (4535 ng/mL), which are more than 190-fold and 3800-fold higher, respectively, the inhibitory concentrations for ROCK1 and ROCK2. However, selectivity against these other kinases and non-kinase molecules, compared to ROCK1 and ROCK2, is unknown as concentrations lower than 0.5 µM or 10 µM were not evaluated.

(b) (4)

The applicant states that administration of RHOPRESSA once daily for 8 days resulted in clinical exposure of netarsudil below the lower limit of quantitation of 0.100 ng/mL, except for one subject with levels of 0.11 ng/mL for the primary metabolite (Section 12.2 of the proposed label). Therefore, it is unlikely that inhibitory activity against ROCK1 and ROCK2 and secondary kinase targets or non-kinase targets would have any detectable systemic pharmacological effect at the intended ocular dosing regimen in humans.
"state."

The IC_{50} for the inhibitory effect of AR-13324 on hERG potassium current was 0.4 µM [equivalent to 181 ng/mL (0.4 µmole/L x 453.54 g/mole = 181 ng/mL)], which is the concentration of netarsudil unbound to plasma proteins that would be needed to reach the IC_{50} *in vivo*. QTc prolongation was not observed in the safety pharmacology study in dogs at doses (based on free base) up to 17.6 mg/kg IV (872 ng/mL) or a 28-day repeat-dose toxicity study in dogs at doses up to 0.53-0.67 mg/kg (51-63 ng/mL). As noted by the applicant, only 3% of netarsudil is expected to be unbound to plasma protein *in vivo* (Study # AR-13324-IPK01). Therefore, low levels of active free drug would explain the lack of QTc prolongation in vivo. As noted above, clinical exposure of netarsudil was generally below the lower limit of quantitation of 0.100 ng/mL at the recommended clinical dose, indicating minimal risk for QTc prolongation to be observed in humans at the intended dosing regimen.

The ocular safety of Netarsudil Ophthalmic Solution was evaluated in rabbits and monkeys in repeat-dose ocular toxicity studies of 7-day, 28-day, 3/6-month (rabbits) and 9-month (monkey) duration. Findings in both species included signs of ocular irritation, (i.e., conjunctival congestion [hyperemia], discharge and/or swelling), corneal alterations, and decreased IOP (Table 21). The severity and extent of these findings were dose dependent. Generally, the signs of ocular irritation and corneal alterations decreased in severity, or reversed, despite continuous dosing.

Table 21: Main Ocular Findings after Topical Ocular Administration of Netarsudil Ophthalmic Solution

Ocular Study	Main findings
9-Month Monkey	Mid (0.02% BID) and high dose (0.04% BID) • Conjunctival hyperemia, chemosis (less often), and/or discharge (rare) – 1st week of dosing (mild) • Reddening of the eye and/or eyelid, as well as partial eye closure – observed occasionally and resolved with continued dosing • Faint (score = 1) or diffuse (i.e., involving the entire cornea) superficial corneal haze - resolved in all but one high-dose animal before Week 39 despite continued dosing; resolved during recovery in the high-dose animal The NOAEL was considered to be the high dose (0.04% BID) based on the transient nature of findings and lack of histologic correlate. NOEL for corneal haze was 0.01% BID.
3/6-Month Rabbit	Mid (0.03% BID) and high dose (0.06% BID) • Clinical signs of increased incidence and severity of conjunctival redness and/or discharge – throughout the study (mild to moderate) – not present during recovery period • Ophthalmoscopy observations of conjunctival redness, discharge, and/or chemosis – 1st 3 months of dosing (mostly mild) All dose groups (0.01, 0.03, and 0.06% BID) • Corneal opacity in one or two animals at all dose levels, with punctate fluorescein uptake (minimal) – Week 13 slit lamp evaluations (reversed by Week 18 evaluations). High dose (0.06% BID) • Microscopic corneal lesions consisting of peripheral vascularization, mixed cell inflammation, attenuation of the overlying corneal epithelium, small ulcer – at 6-month sacrifice (not present at interim or recovery sacrifice) The NOAEL was considered to be the mid dose (0.02% BID), based on microscopic findings in the corneal at the high dose.
1-Month Monkey	Mid (0.04% BID) and high dose (0.06% BID) • Redness around the eye, periorbital swelling, and/or ptosis – did not persist with continued dosing • Corneal haze – persisted throughout the dosing period but resolved during recovery period High dose (0.06% BID) • Microscopic corneal findings of unilateral hyperplasia in the corneal epithelium (minimal) – not present in recovery animals The NOAEL was the low dose, 0.02% BID.
28-Day Rabbit	Mid (0.04% BID) and high dose (0.06% BID) • Conjunctival redness and discharge (minimal) - diminished incidence with continuous dosing – not present during recovery period • Conjunctival congestion, swelling, and/or discharge (slight) • Microscopic corneal lesions consisting of slight degeneration/erosion – not present in recovery animals All doses groups (0.02, 0.04, or 0.06% BID) • Corneal findings (focal opacities, punctate ulcers) throughout the study – not present on Day 28 (last day of dosing) The NOAEL was the low dose, 0.02% BID.
7-Day Monkey	Initial study: Mid (0.04% QID) and high dose (0.12% QID) • Reddened conjunctiva, eye discharge, reddened sclera, and/or swollen conjunctiva - between Day 1 and Day 7 - reversible

	• Corneal cloudiness (Grade 1-2) with or without corneal edema at ≥0.04% QID – persisted during 7-day recovery period • Microscopic findings in the cornea (minimal to moderate hypertrophy/hyperplasia/ edema/apoptosis), eyelids (minimal to mild hypertrophy/hyperplasia of the conjunctival epithelium/inflammatory cell subconjunctival infiltrates), and nasolacrimal ducts (mild mononuclear cell or neutrophil infiltrates/edema) – corneal findings still present at recovery but of lower severity (minimal-mild) The NOAEL was the low dose, 0.01% QID. Follow up study: 0.02% BID, 0.04% QD and BID, and 0.08% QD • Conjunctival congestion and swelling - all dose levels, grade 1, incidence decreased on Day 7 • Microscopic findings of corneal epithelial hyperplasia/hypertrophy, hypertrophy/hyperplasia of the conjunctival epithelium and/or epidermis – minimal to mild The NOAEL was the low dose, 0.02% BID or 0.04% QD.
7-Day Rabbit	High dose (0.04% QID) • Severe ocular irritation during the first few days of treatment – lessened in both severity and incidence with continued dosing • Corneal epithelial damage – resolved during recovery period • No microscopic findings in the cornea The NOAEL was 0.08% QD, based on the severe ocular irritation and high incidence of corneal staining at the high dose.

In monkey, plasma levels of the test article and its metabolites were generally below the limit of quantitation (1 ng/mL). In rabbits, systemic exposure of netarsudil and metabolites AR-13503 was minimal (below or near the lower limit of quantitation of 1 ng/mL). Consistent with the low systemic exposure, no systemic toxicities were observed in the repeated-dose ocular toxicity studies.

The exposure margins for ocular findings at the NOAEL observed in the chronic ocular toxicity studies are shown in the following table:

Table 22: Exposure Margins at Ocular NOAEL

Species	NOAEL (mg/kg)	Drop vol (µL)	NOAEL (mg/eye)	Exposure Margin[a] (0.02% QD; (b) (4) mg/eye)
Monkey				
9 months	0.04% BID	35	0.056	8
	0.01% BID (NOEL)[b]	35	0.007	1
Rabbit				
3/6 months	0.02% BID	35	0.014	2

[a]Based on a drop volume of 35 µL
[b]No observed effect level for corneal haze.

Findings of conjunctival hyperemia, conjunctival hemorrhage, and erythema of the eyelids were observed in the Phase 3 studies. The applicant claims that conjunctival hyperemia was the most common adverse event. This finding could be related to the

pharmacological effect of dilatation of blood vessels caused by the ability of Rho kinase inhibitors to relax vascular smooth muscle. This reviewer agrees with the applicant that conjunctival hyperemia (without signs of ocular irritation) is not a toxicological concern, but can be a cosmetic concern for some patients. As noted by the applicant, the adverse events of conjunctival hemorrhage and erythema of the eyelid may also be related to the vasoactive effects of Rho kinase inhibition.

The NOEL for corneal haze provides an exposure margin of 1 for the intended clinical dose. The finding of corneal haze in the 9-month monkey study was further described in the study report as "multiple, very fine, pinpoint depositions or droplets in the superficial cornea". Netarsudil is a cationic amphiphilic drug (CAD), which are known to induce phospholipidosis. In a cell-based assay for drug-induced phospholipidosis (Section 10. Special Toxicology Studies), netarsudil was determined to induce phospholipidosis with an EC_{50} of 1.1μM (498.88 ng/mL), which is a physiological concentration for netarsudil in the cornea based upon rabbit ocular PK studies.

As noted by the applicant, it is possible that the finding of corneal haze in monkeys is related to the findings of "corneal deposits" and "corneal verticillata" associated with Netarsudil Ophthalmic Solution 0.02% treatment in Phase 3 clinical studies. The applicant also noted that if corneal haze in monkeys is due to phospholipidosis, it is worth noting that the timing with which it arises and resolves is faster than what has been seen in the Phase 3 clinical studies. The applicant indicated that an observational study (AR-13324-OBS01) of subjects who developed corneal deposits in the pivotal Phase 3 trials is being conducted to assess changes in corneal deposits over time and effects in visual function. The results will be filed in the NDA 4-month safety update. As the corneal haze resolved in monkeys despite continued dosing (all but one animal), or during the recovery period (one animal), this proposal is considered acceptable from the nonclinical perspective.

The systemic toxicity profile of netarsudil was evaluated in rats and dogs in repeat-dose studies of 7-day and 28-day duration by the intravenous route. Based on the result of the systemic toxicity studies in rats and dogs, as well as the cardiovascular safety pharmacology study in telemetered dogs, common findings included cardiovascular (vasodilation) effects and inflammation at the site of injection. The vasodilation was apparent by the red discoloration observed in the skin of various body surfaces (e.g., ears, legs, abdomen, and chest) and eye (sclera) and decreased in blood pressure associated with increased heart rate. These are expected effects based on the pharmacological activity of Rho-kinase inhibitors.

Additional targets observed at the highest doses tested in the early short-term (7-day) repeat-dose IV toxicity studies in rats and/or dogs included the hematopoietic system (RBC parameters and WBC types alterations, bone morrow and lymph nodes microscopic findings), blood coagulation system (increased PT and/or APTT) and several organs (Table 8); with clinical chemistry changes consistent with these targets. These findings were not observed at the lower doses evaluated in the 28-day repeat-dose IV toxicity studies in rats (0.04-0.74 mg/kg) and dogs (0.04-0.67 mg/kg).

The doses associated with these findings and related systemic exposure (C_{max} and AUC) are shown in Table 23. Based on the observed clinical exposure of netarsudil generally below the lower limit of quantitation of 0.100 ng/mL at the recommended clinical dose, it is unlikely these findings would have significant clinical relevance.

Table 23: Systemic Targets and Exposure at NOAEL

Toxicity	Species	Study	NOAEL (mg/kg)	C_{max} (ng/mL)*	AUC (ng•hr/mL)*
Decreased blood pressure; increased hear rate	Dog	Safety Pharm	0.7	27	---
Red skin on various body surfaces	Rat	7-day IV	1	14	62
	Dog	7-day IV	1	43	264
Inflammation at injection site (tail)	Rat	7-day IV	1	14	62
	Dog	7-day IV	1	43	264
	Dog	28-day IV	0.18	14	82
Mortalities	Dog	7-day IV	1	43	264
Alterations in RBC parameters, increased prothrombin time or APTT	Rat	7-day IV	1	14	62
	Dog	7-day IV	1	43	264
Alterations in WBC types	Rat	7-day IV	3	54	240
	Dog	7-day IV	1	43	264
Abnormal erythrocyte morphologies	Rat	7-day IV	3	54	240
	Dog	7-day IV	1	43	264
Alterations in clinical chemistry parameters	Rat	7-day IV	1	14	62
	Dog	7-day IV	1	43	264
Increased incidence of hepatic extramedullary hematopoiesis	Rat	7-day IV	1	14	62
Microscopic findings in heart, kidneys, lungs, gallbladder, adrenal gland, bone marrow, urinary bladder thymus	Dog	7-day IV	1	43	264

*Average of male and female mean values on Day 7 or 28, depending on study duration.

Embryofetal development toxicity studies in both rats and rabbits showed dose dependent increases in embryofetal lethality such as increased early resorptions, increased in post-implantation loss, decreases in litter size, decreased number of mothers with viable fetuses, and decreased number of viable fetuses.

Signs indicative of abortions were observed in rats. At the high dose (3 mg/kg/day), none of the 24 gravid rats had viable fetuses, and at the mid dose (0.3 mg/kg), 16 of the 24 gravid rats had viable fetuses, compared to gravid controls which all had viable fetuses at C-section. Offspring malformations were limited to a slight increase in the mean number of ossified caudal vertebrae at ≥0.1 mg/kg/day and forelimb phalanges at ≥0.03 mg/kg/day.

In rabbits, abortion was not observed in the definitive study at concentrations up to 5 mg/kg/day. A confirmed abortion and a probable abortion occurred in the dose-range-finding study at 5 mg/kg/day. The relationship to treatment is not clear, as no abortions were noted at a high dose of 9 mg/kg/day in the same study. A slight decreased fetal weight was noted at 5 mg/kg/day in the dose-range-finding as well as the definitive study. The study report states that there were no netarsudil (AR-13324)-related fetal external, soft tissue, or skeletal fetal malformations or variations at any dose. However, there were some findings with higher incidence in test-article treated groups. These included umbilical hernia and thoracogastroschisis in one high-dose (5.0 mg/kg/day) and one mid-dose (3.0 mg/kg/day) fetus, respectively; and increased number of fetuses with an absent intermediate lung lobe at 5.0 mg/kg/day. Skeletal examination confirmed the abdominal wall defects noted in fetus in the 3.0 mg/kg/day dose group (thoracogastroschisis), which presented as a duplicated manubrium and sternal centra at skeletal exam. This fetus also had an incompletely ossified manubrium, incompletely ossified sternal centra, and incompletely ossified pubes.

Although the umbilical hernia and thoracogastroschisis were observed in only two fetuses (in 2 litters), these are rare findings. In addition, the incidence was above the historical control range reported in Charles River Historical Control database (June 2008-2010) (note: the applicant did not included performing laboratory historical control data with the NDA). Therefore, a test article-related effect in abdominal wall defects cannot be ruled out. As noted above (Section 9.2 Fertility and Embryonic Development), cursory review of the literature revealed existing evidence supporting a role for Rho/Rho-kinase signaling pathway in branching morphogenesis in the fetal lung in mice and rats. Therefore, a test article related effect in the increased number of fetuses with absent intermediate lung lobe cannot be ruled out.

The NOAEL for both maternal and embryofetal development toxicity was considered to be 0.1 mg/kg/day in the rat. In the rabbit, the NOAEL for maternal toxicity was 5 mg/kg/day, whereas the NOAEL for embryofetal development toxicity was 0.5 mg/kg/day. The systemic exposure (C_{max} and AUC) at these doses on GD 17 (rats) or GD 19 (rabbits) are shown in Table 24. Based on the observed clinical exposure of netarsudil generally below the lower limit of quantitation of 0.100 ng/mL at the recommended clinical dose, there is low risk for embryofetal toxicity to be observed at the intended dosing regimen.

Table 24: Exposure Margins - Embryofetal Toxicity

Species	NOAEL (mg/kg)	C_{max} (ng/mL)*	$AUC_{0\text{-}24hrs}$ (ng•hr/mL)*	Exposure Margin* (LLOQ = 0.100 ng/mL)
Rat				
Mother	0.1	4.07	3.368	>40
Offspring	0.1	4.07	3.368	>40
Rabbit				
Mother	5	148	180	>1480
Offspring	0.5	21.4	23.4	>214

*Human systemic exposure of netarsudil was below the lower limit of quantitation (LLOQ = 0.100 ng/ml).

The full battery of genetic toxicity studies did not show netarsudil has mutagenic or clastogenic potential. Carcinogenicity studies were not conducted. The justification provided by the applicant to omit these studies was considered acceptable (see Section 8. Carcinogenicity).

In conclusion, the nonclinical data presented in this NDA provides adequate safety support for the intended dosing regimen of Netarsudil Ophthalmic Solution 0.02% once daily ((b) (4) mg/eye) for the indication of reduction of IOP. This reviewer recommends approval of the NDA.

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/s/

MARIA I RIVERA
11/06/2017

LORI E KOTCH
11/06/2017

PHARMACOLOGY/TOXICOLOGY NDA FILING CHECKLIST

NDA Number: 208254 **Applicant: Aerie Pharmaceuticals, Inc** **Stamp Date: 2-28-2017**

Drug Name: Rhopressa™ (Netarsudil Ophthalmic Solution) 0.02% **NDA/BLA Type: Commercial**

Note: This NDA is a resubmission after withdrawal. The only nonclinical information included in the resubmission is Section 2.4 Nonclinical Overview. The filing review is based on nonclinical information submitted in the original submission.

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required and requested IND studies in accord with 505 (b)(1) and (b)(2) including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		Aerie submitted a waiver request for carcinogenicity studies.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		

PHARMACOLOGY/TOXICOLOGY NDA FILING CHECKLIST

	Content Parameter	Yes	No	Comment
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	X		The applicant included the required nonclinical sections. The applicant claims the API is not absorbed systemically following topical ophthalmic application. Therefore, no nonclinical data was included in Section 8 of the label. As noted above, the sponsor is requesting a waiver for carcinogenicity assessment.
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)	X		A definitive response is pending CMC review.
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?			N/A

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.
None has been identified.

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/s/

MARIA I RIVERA
03/30/2017

LORI E KOTCH
03/31/2017

PHARMACOLOGY/TOXICOLOGY NDA FILING CHECKLIST

NDA Number: 208254 **Applicant: Aerie Pharmaceuticals, Inc** **Stamp Date: 8-30-16**

Drug Name: Rhopressa™ (Netarsudil Ophthalmic Solution) 0.02% **NDA/BLA Type: Commercial**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required and requested IND studies in accord with 505 (b)(1) and (b)(2) including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		Aerie submitted a waiver request for carcinogenicity studies.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		

PHARMACOLOGY/TOXICOLOGY NDA FILING CHECKLIST

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	X		The applicant claims the API is not absorbed systemically following topical ophthalmic application. Therefore, no nonclinical data was included in Section 8.2 of the label. As noted above, the sponsor is requesting a waiver for carcinogenicity assessment.
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)	X		A definitive response is pending CMC review.
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?			N/A

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.
None has been identified.

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/s/

MARIA I RIVERA
10/03/2016

LORI E KOTCH
10/03/2016