CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

217603Orig1s000

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:	217603
Supporting document:	SN0001
Applicant's letter date:	August 25, 2022
CDER stamp date:	August 25, 2022
Product:	Xdemvy (Lotilaner Ophthalmic Solution, 0.25%)
Indication:	Demodex blepharitis
Applicant:	Tarsus Pharmaceuticals, Inc.
Review Division:	Ophthalmology
Reviewer:	Muriel Saulnier, DVM, PhD, DABT
Supervisor/Team Leader:	Kimberly Hatfield, PhD (acting for Lori Kotch, PhD, DABT)
Division Director:	Wiley Chambers, MD
Project Manager:	Dheera Semidey

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA217603 are owned by Tarsus Pharmaceuticals, Inc. or are data for which Tarsus Pharmaceuticals, Inc. have obtained a written right of reference.

Any information or data necessary for approval of NDA217603 that Tarsus Pharmaceuticals, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA217603.

TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

Tarsus Pharmaceuticals, Inc. (Tarsus) is submitting a New Drug Application (NDA) for XDEMVY™ (Lotilaner Ophthalmic Solution, 0.25%) seeking the 505(b)(1) pathway for approval. XDEMVY is indicated for the treatment of Demodex blepharitis.

The applicant describes blepharitis as a disease characterized by inflammation of the eyelid margins. Subjects with blepharitis often experience red and watery eyes, burning or stinging in the eyes, itchy, red, and/or swollen eyelids, and a crustiness around the eyelashes; blepharitis can also lead to abnormal growth or loss of eyelashes. Blepharitis is commonly associated with infestation of eyelash follicles and meibomian glands by 2 species of microscopic obligate parasitic mites: Demodex folliculorum or Demodex brevis, respectively. There are no approved pharmaceutical treatments for Demodex blepharitis.

The recommended dosage of XDEMVY is one drop to be instilled in each eye (OU) twice daily (BID) (approximately 12 hours apart) for 6 weeks. The therapeutic dose is (b) (4) mg/eye/BID/OU assuming a (b) (4) µL volume drop equivalent to (b) (4) mg/eye/day or (b) (4) mg/kg for a 60 kg body weight.

Lotilaner Ophthalmic Solution, 0.25% is a combination product comprised of a drug constituent and a container-closure system that includes the bottle, tip, and cap. The review of the device is deferred to CDRH.

Lotilaner has been previously developed as an oral veterinary drug for the control of flea and tick infestations in dogs and cats (brand name Credelio™; NADA 141-494, NADA 141-528). Tarsus Pharmaceuticals has acquired a license from Elanco Animal Health to develop Lotilaner for human use.

1.2 Brief Discussion of Nonclinical Findings

Lotilaner is an ectoparasiticide and insecticide initially developed as a veterinary product for control of flea and tick infestations and has also been found effective against Demodex mites. The nonclinical safety of Lotilaner and Lotilaner Ophthalmic Solution, 0.25% were evaluated in the rabbit, rat and dog which were considered appropriate.

In the preclinical development studies, Lotilaner is also referred to as TP-03, IOB920 and AHC2224920 as the same active compound.

Pharmacology
Key Findings:

- Isoxazolines, including Lotilaner, are specific inhibitors of (GABA)-gated chloride channels (GABACls) paralyzing the nervous system of insects or arachnids leading to their death. Lotilaner is not an inhibitor of mammalian GABACls when tested at up to 30 µM (18 µg/mL) *in vitro*.

- Proof of concept studies were not conducted for the indication of Demodex blepharitis.

- Lotilaner was evaluated for off-target activity *in vitro* at a concentration of 10 µM (6 µg/mL) against a comprehensive panel of enzymes, ion channels, and receptors. There were no clinically relevant findings.

Safety Pharmacology:
Key Findings:
The central nervous system, respiratory and cardiovascular assessments of Lotilaner were evaluated in GLP studies *in vitro* (hERG assay) and *in vivo* in rats and dogs. No clinically relevant adverse effects were noted in any of the studies.

PK/ ADME/ TK:
Key Findings:
Absorption
In Dutch belted rabbits dosed twice daily for 8 days consecutively with (b) (4) mg/eye/day/OU Lotilaner Ophthalmic Solution, 0.25%, the test article accumulated up to 7-fold in the blood after multiple administrations with mean C_{max} observed at 6 h post-dose. On Day 8, the systemic exposure decreased steadily through 10 days post last dose. Lotilaner $T_{1/2}$ in blood was over 2 days (> 57 h) with an AUC_{last} of 617 h.ng/mL and an apparent volume of distribution consistent with large tissue distribution of the drug product.

In the ocular tissues, lotilaner $T_{1/2}$ was over 11 days (up to 274 h) consistent with the accumulation of the test compound after multiple exposures compared to a single exposure. The average C_{max} was the highest in the eyelid margins and palpebral conjunctiva followed by the cornea, bulbar conjunctiva, lachrymal glands, and the iris/ciliary body. Overall, the data indicated that inferior and superior eyelid margins were prone to drug accumulation, coupled with the lowest clearance in these tissues and an AUC_{inf} 10-fold higher than in all other ocular tissues. Low clearance was also found in the tears that may also be prone to test article accumulation.

Distribution
In vitro, Lotilaner at 5 µM (3 µg/mL) was highly plasma protein bound (≥ 99%) in all species studied including humans. In rats and dogs after oral exposure to Lotilaner, tissue distribution occurred mainly in liver > fat > muscle.

Metabolism
In vitro, Lotilaner was metabolically stable in hepatocytes from mouse, rat, rabbit, dog, monkey, and human. This was confirmed *in vivo* in rats and dogs. Negligible amounts of metabolites were identified in blood, urine, and tissue samples with the parent compound being predominant. Metabolite profiling was not conducted in any species including humans due to the observed *in vitro* hepatocyte stability and the absence of liver metabolism.

Excretion
The excretion of unchanged product in rats and dogs after oral exposure was mostly fecal with a few percentages recovered in the urine. A cat study with Lotilaner by the oral route indicated bile excretion of unchanged product, reabsorption, and recirculation that may explain the long $T_{1/2}$ and accumulation of Lotilaner in tissues observed in the animal studies.

Based on the metabolism and excretion results presented herein, this reviewer anticipated there was no disproportional or unique metabolite(s) in humans compared to the species used for toxicity studies, i.e., the rat, the rabbit, and the dog.

<u>Ocular and Systemic Toxicity</u>
Key Findings:

- In two ocular GLP toxicity studies with Lotilaner Ophthalmic Solution, 0.25%, in the Dutch Belted rabbit and in the Beagle dog for 6 and 9 months, respectively, the no observed adverse effect level (NOAEL) was the highest dose tested of (b) (4) µg/eye/day/OU, i.e., (b) (4) µg/animal/day. There were no test-article related findings at any dose in either species.

  In the rabbit, at the NOAEL, mean C_{max} and AUC_{0-24} values on Day 182 were 251 ng/mL and 5,385 ng*hr/mL, respectively, in males and females combined (no gender differences in exposure). In the dog, at the NOAEL, mean C_{max} and AUC_{0-24} values on Day 273 were 403 ng/mL and 7,790 ng*hr/mL, respectively, in males and females combined (no gender differences in exposure).

  Ocular safety margins to the clinical dose are 2.66X in both rabbit and dog, based on direct dose comparison, while systemic safety margins are (b) (4) X with the rabbit

and (b)(4)X with the dog, based on mg/m^2 equivalent. These safety margins were found to be acceptable by this reviewer.

This reviewer's comment: The histopathology of the nasal cavities was not assessed in any of the ocular toxicity studies. The nasal turbinate and nasopharynx are important target organs of toxicity after topical, ocular administration, and should have been examined.

- The systemic toxicity of AHC 2224920 was assessed by daily oral exposure for 13 weeks in the rat and once *per* month for 8 months in the Beagle dog in a GLP fashion. Test-article related microscopic findings were noted in body weight, skin/mucosa, ovary, lung, adrenal, pituitary (males only) and thymus in rats. Mortality due to early sacrifice of moribund animals occurred at 60 mg/kg/day in female rats and at ≥40 mg/kg/day in male rats. However, none of the findings were clinically relevant as they occurred at doses > 500X the therapeutic dose in mg/m^2 equivalent.

  There were no adverse findings in the dog up to the highest dose tested of 215 mg/kg which corresponded to a systemic safety margin > 20,000X with the therapeutic dose in mg/m^2 equivalent. Considering the exposure to Lotilaner was relatively constant in the dog study by oral exposure, this reviewer found the design of monthly administration in the dog acceptable for assessing systemic toxicity in the context of a daily ocular exposure in humans for 6 weeks. In these systemic studies, at the NOAELs in the male rat of 20 mg/kg/day and in the female rat of 40 mg/kg/day, Day 91 C_{max} and $AUC_{0\text{-}24hr}$ values achieved 56 µg/mL and 1101 µg *hr/mL, respectively, at 20 mg/kg/day and 97 µg/mL and 1879 µg*hr/mL, respectively, at 40 mg/kg/day, for males and females combined (no gender differences in exposure). At the NOAEL in the dog of 215 mg/kg, mean Month 8 C_{max} and $AUC_{0\text{-}672hr}$ values achieved were 21.3 µg/mL and 9870 µg *hr/mL, respectively, for males and female combined (no gender differences in exposure).

Genotoxicity

Key Findings:

AHC 2224920 was negative for genotoxic potential in a complete battery of genotoxicity GLP studies (i.e., Ames test, *in vitro* chromosome aberration test in cultured human lymphocytes and *in vivo* micronuclei assay in rats).

Carcinogenicity

Key Findings:

No studies were performed. The Sponsor provided a rationale for not conducting carcinogenicity studies with Lotilaner that was found to be acceptable by this reviewer (see "Carcinogenicity Section").

Reproductive Toxicity

Key Findings:

- In an embryo-fetal toxicity in the rat with AHC 2224920 by oral gavage at doses of 9, 18 and 50 mg/kg/day, the NOAEL for maternal and fetal toxicities was 18 mg/kg/day providing a safety margin based on dose of (b) (4) X (in mg/m^2 equivalent) with the Maximum Recommended Human Ophthalmic Dose (MRHOD) of (b) (4) mg/kg for a 60 kg human. Above NOAEL, the test article was not tolerated resulting in marked body weight loss and early termination of multiple females at 50 mg/kg. There was a marked effect on mean fetal weight at 50 mg/kg/day and there was an indication of a developmental delay characterized by an increased number of fetuses with incomplete ossification of individual bones of the skeleton. A test article related malformation of situs inversus of the thoracic and abdominal viscera was observed in 1 fetus from this group. No TK data were produced in this study.

- In an embryo-fetal toxicity in the rabbit with AHC 2224920 by oral gavage at doses of 2.5, 7.5 and 20 mg/kg/day, the maternal NOAEL was 7.5 mg/kg/day (C_{max} = 3,200 ng/mL; AUC_{0-24} = 56,200 h*ng/mL) and the fetal NOAEL was 20 mg/kg/day (C_{max} = 8,680 ng/mL; AUC_{0-24} = 170,000 h*ng/mL). These NOAELs provided safety margins based on dose of (b) (4) (in mg/m^2 equivalent) for maternal and fetal toxicities, respectively, considering a MRHOD of (b) (4) mg/kg for a 60 kg human. Decreased food consumption during the dosing phase was noted for animals administered 20 mg/kg/day. While the magnitude of reduced mean food consumption was small, one animal in this group demonstrated severely reduced food consumption accompanied by body weight loss requiring unscheduled moribund sacrifice. It is the opinion of this reviewer that the demise of this animal was test-compound related. In all other animals, except for food consumption that was altered at 20 mg/kg/day, no Lotilaner-related effects were observed on clinical observations, mean body weight, macroscopic observations, reproductive performance, cesarean section parameters, or fetal evaluations at any dose level.

- In a 2-generation study in the rat with AHC 2224920 by oral gavage at doses of 1, 5 and 40→20 mg/kg/day administered to the parental generation (F0), the NOAEL for maternal and fetal toxicities was 5 mg/kg/day equivalent to a safety margin based on dose of (b) (4) X (in mg/m^2 equivalent) with the MRHOD of (b) (4) mg/kg for a 60 kg human. Females at 40→20 mg/kg/day showed reduced pregnancy rate

and low implantation rates, both associated with low body weight gain and low food consumption. No F1 generation could be produced at 40→20 mg/kg/day due to test article related decreased fertility of the females in the F0 generation. At 1 and 5 mg/kg/day mating and fertility of the F0 generation was unaffected. At 5 mg/kg/day, males in the F1 generation had slightly increased mean body weights compared to control and their adjusted testis weights were slightly increased accompanied by increased sperm velocity that were not considered adverse since mating and fertility of the F1 generation was unaffected. Slightly lower mean body weights during lactation of the F2 pups at 5 mg/kg/day were associated with a slight delay in the attainment of righting reflex and pinna detachment, but later development during weaning was normal. No TK data were produced in this study.

- There was no designated fertility study, however the results of the 2-generation study in the rat suggested that the NOAEL for male fertility was 20 mg/kg/day under the conditions of the study, which provided a safety margin based on dose of (b) (4) X (in mg/m^2 equivalent) with the MRHOD of (b) (4) mg/kg for a 60 kg human.

Special Studies
Key Findings:

- AHC 2224920 was minimally irritating to the eye after instillation of 100 mg into the conjunctival sac of male New Zealand White rabbits in a 3-day ocular irritation test.

- AHC 2224920 triggered no alert for respiratory and skin sensitization after *in silico* analysis. The absence of skin sensitization was confirmed with a Local Lymph Node Assay in the mouse.

- AHC 2224920 was classified as a non-irritant for the skin based on the Draize classification system in the rabbit.

- In an acute dermal toxicity test with rats, the median lethal dose of AHC 2224920 exceeded 2000 mg/kg.

1.3 Recommendations

1.3.1 Approvability

The clinical dose ((b) (4) mg/eye/day) and the length (6 weeks) of the therapeutic exposure were supported by 2 ocular and 2 systemic toxicity studies of adequate length

and performed under GLP conditions. Safety pharmacology and genotoxicity batteries were complete and acceptable as were embryofetal toxicity studies in 2 species, i.e., the rat and the rabbit, and a 2-generation reproductive toxicity study in the rat to cover both male fertility and reproductive and developmental toxicity of the product. The request for lack of carcinogenicity studies was also justified. Nasal turbinate and nasopharynx were not examined under the microscope in any of the ocular studies which was a drawback to the complete evaluation of the potential ocular toxicity of Lotilaner Ophthalmic Solution, 0.25%, but does not influence the decision on approvability of this product. No clinical signs were observed in any animal study that could suggest nasal cavities and/or throat irritation.

It is the opinion of this reviewer that the product is approvable from a pharmacology and toxicology perspective.

1.3.2 Additional Non-Clinical Recommendations

Histopathology was not performed on the nasal cavities and nasopharynx in any ocular toxicity studies, hence the potential irritative effect of the test compound on these tissues is unknown. Caution is recommended in the clinic.

1.3.3 Labeling

The proposed edits to product labeling are addressed under a separate review.

2 Drug Information

2.1 Drug

CAS Registry Number
1369852-71-0

Generic Name
Lotilaner Ophthalmic Solution, 0.25%

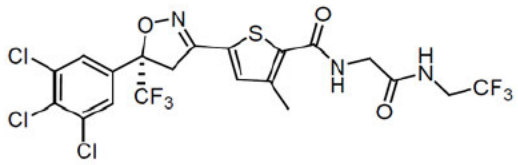
Other Names
TP-03; lotilaner; AHC2224920; (b) (4).

Proposed tradename
XDEMVY

Chemical Name
2-Thiophenecarboxamide, 5-[(5S)-4,5-dihydro-5-(3,4,5-trichlorophenyl)-5-(trifluoromethyl)-3- isoxazolyl]-3-methyl-N-[2-oxo-2-[(2,2,2-trifluoroethyl) amino] ethyl]-2-thiophenecarboxamide.

Molecular Formula/Molecular Weight
$C_{20}H_{14}Cl_3F_6N_3O_3S$/596.76 g/mol

Structure or Biochemical Description
S-enantiomer



Pharmacologic Class
Ectoparasiticide, insecticide

2.2 Relevant INDs, NDAs, BLAs and DMFs

Veterinary drug Credelio™; NADA 141-494, NADA 141-528

IND143686

In module 3 of this NDA, reference is made to the following Drug Master Files (DMFs) and letters of authorization were provided for:

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

2.3 Drug Formulation

XDEMVY (Lotilaner Ophthalmic Solution, 0.25%) is a sterile, preserved, multi-dose ophthalmic solution containing lotilaner, 0.25%, as the active ingredient. It is preserved with potassium sorbate and contains the following additional inactive ingredients: Edetate disodium, Hypromellose (HPMC (b) (4) Polyoxyl 35 castor oil (b) (4), Glycerin, Dibasic sodium phosphate, Monobasic sodium phosphate, and Water for Injection (Table 1).

Table 1: Qualitative and Quantitative Composition of XDEMVY

Component	Reference to Quality Standard	Function	Concentration (mg/ mL)	Concentration %w/v or (g/100 mL)
Lotilaner	In-house	Active	2.5[1]	0.25
Potassium Sorbate	USP/NF[2]	Preservative	(b) (4)	(b) (4)
Edetate Disodium	USP/NF	(b) (4)		
Hypromellose (HPMC (b) (4))	USP/NF			
Polyoxyl 35 Castor Oil (b) (4)	USP/NF			
Glycerin	USP			
Dibasic Sodium Phosphate (b) (4)	USP			
Monobasic Sodium Phosphate (b) (4)	USP			
Water for Injection	USP			

[1] (b) (4)

[2] Potassium sorbate that complies with the testing requirements per USP/NF

(Copied from Module 2.3.P Drug Product [Lotilaner Ophthalmic Solution, 0.25%] on p 5)

XDEMVY is a combination product comprised of a drug constituent and a device constituent part. The device constituent (i.e., container-closure system (CCS)) of the Lotilaner Ophthalmic Solution, 0.25% drug product includes the bottle, tip, and cap. XDEMVY will be available in two presentations (i.e., commercial presentation: 10 mL fill volume in a 11 mL bottle and physician sample presentation: 1.5 mL fill volume in a 3 mL bottle).

2.4 Comments on Novel Excipients

The vehicle for Lotilaner Ophthalmic Solution, 0.25%, used in all pivotal ocular GLP toxicity studies was the clinical formulation which qualified the excipients.

2.5 Comments on Impurities/Degradants of Concern

Drug product

The acceptance criteria included in the drug product specification were established in accordance with ICH Q6A[1] and with ICH Q3B(R2)[2].

---

[1]https://www.federalregister.gov/documents/2000/12/29/00-33369/international-conference-on-harmonisation-guidance-on-q6a-specifications-test-procedures-and
[2]https://www.fda.gov/media/71733/download

Lotilaner-related substances or degradation products present in Lotilaner Ophthalmic Solution, 0.25%, are Lotilaner R enantiomer, a drug substance process impurity, and four potential drug product degradation products which are unidentified impurities seen at approximate relative retention times (RRT) of (b) (4). These unidentified impurities are observed at levels well below the threshold for identification and structural characterization as prescribed in ICH Q3B(R2), Impurities in New Drug Products.

Lotilaner Ophthalmic Solution, 0.25% has undergone evaluation for potential container/closure leachables impurities. The Analytical Evaluation Threshold (AET) has been estimated to be approximately (b) (4) ppm based on the PQRI guidance and using the exposure dose of (b) (4).

Tarsus also has estimated the AET to be (b) (4) ppm based on concentration. Unidentified leachables (alternatively referred to as leachable impurities) above the (b) (4) AET ((b) (4) ppm) have been observed in drug product stability batches but consistently below the commonly acceptable identification threshold of (b) (4) ppm suggested by the Agency for monitoring leachables in topical ophthalmic products. Tarsus will continue to monitor potential leachables through proposed shelf life and will identify leachables, if levels, consistently exceed (b) (4) ppm.

Assessments for potential presence of (b) (4), elemental impurities and (b) (4) impurities were also conducted. As no risks were identified, (b) (4), elemental impurities and (b) (4) impurities are not specified for the drug product.

Drug substance
The acceptance criteria included in the drug substance specification were established in accordance with ICH Q1A(R2)[3], ICH Q3A(R2)[4], ICH Q3C(R8)[5], ICH Q3D(R2)[6] and ICH Q6A[1].

The impurities in the drug substance are:

- R-Lotilaner enantiomer, other specified, unspecified, process-related (b) (4)) and potentially genotoxic organic impurities. All impurities either satisfied ICH Q3A[4] thresholds or were found absent in analyzed batches ((b) (4)). The potential genotoxic impurities were predicted to be (b) (4) below the ICH M7 TTC limit of 1.5 µg / day.
- Residual solvents (including (b) (4)). All impurities satisfied ICH Q3C(R8)[5] thresholds.
- Inorganic impurities. All impurities satisfied ICH Q3D(R2)[6] thresholds.

A risk assessment for (b) (4) was completed following the FDA Guidance for Industry: (b) (4) were not identified in Lotilaner drug substance.

---

[3]https://www.fda.gov/media/71707/download
[4]https://www.fda.gov/media/71727/download
[5]https://www.fda.gov/media/138334/download
[6]https://www.fda.gov/media/148474/download
[7] (b) (4)

Further review of the characteristics and specifications of the drug product and drug substance is deferred to CMC.

2.6 Proposed Clinical Population and Dosing Regimen

Proposed labeling in Module 1.1.4

(b) (4)

(b) (4)

2.7 Regulatory Background

Tarsus had several interactions with the Agency prior to the submission of their NDA:

- A type B pre-IND meeting held on June 4, 2019, with meeting minutes archived in DARRTS on 06-26-2019[8], to discuss the generalities of their clinical and nonclinical development plans in support of the proposed indication.
- A type C pre-IND meeting held on December 8, 2020, with meeting minutes archived in DARRTS on 12-21-2020[9], to further discuss their development plan including their proposed waiver of carcinogenicity studies request and the embryofetal development toxicity studies. A carcinogenicity waiver request along with rationale/justification and supporting data is provided in this NDA in line with the Division's previous request (as part of the December 8, 2020, Type C meeting) to submit it with the marketing application.
- A type C pre-IND meeting held on April 20, 2022, with meeting minutes archived in DARRTS on 05-18-2022[10], to discuss the clinical and CMC requirements for the planned NDA submission.
- A pre-NDA meeting held on June 22, 2022, with FDA preliminary comments archived in DARRTS on 06-15-22[11], to discuss further and agree on CMC, preclinical and clinical requirements for an NDA submission. The Sponsor was satisfied with the Agency's responses and required only several clarifications while the meeting was cancelled by the Sponsor.

---

[8]https://darrts.fda.gov/darrts/faces/ViewDocument?documentId=090140af805007db
[9]https://darrts.fda.gov/darrts/faces/ViewDocument?documentId=090140af805bd9b8
[10]https://darrts.fda.gov/darrts/faces/ViewDocument?documentId=090140af8066189e
[11]https://darrts.fda.gov/darrts/faces/ViewDocument?documentId=090140af8066a526

3 Studies Submitted

3.1 Studies Reviewed

(b) (4)*200302: Functional characterization of 4 compounds on 7 different $GABA_A$ receptors subtypes from human and dogs in xenopus oocytes*

(b) (4)*095-0018934: In Vitro Pharmacology Study of Lotilaner*

21-TAR-001: Repeat dose ocular pharmacokinetics of a 0.25% lotilaner topical ophthalmic formulation TP-03 in the Dutch-belted rabbit

21-TAR-002: Single dose ocular pharmacokinetics of a 0.25% lotilaner topical ophtalmic formulation TP-03 in the Dutch-belted rabbit

8445531: Metabolism of Lotilaner in Mouse, Rat, Rabbit, Dog, Monkey, and Human Primary Hepatocytes

8445547: Identification of Human Drug Metabolizing Enzymes Involved in the Metabolism of Lotilaner

(b) (4)*-14-055: ADME study of [^{14}C]-AHC2224920 after intravenous and oral administration in cats*

8443928: In Vitro Plasma Protein Binding and Blood-to-Plasma Partitioning of Lotilaner in Mouse, Rat, Dog, and Human

1020-7114: Lotilaner: A 6-Month Repeat-Dose Topical Ocular Toxicity Study Followed by a 4-Week Recovery Period in Dutch Belted Rabbits

1020-7122: Lotilaner: A 9-Month Repeat-Dose Topical Ocular Toxicity Study Followed by a 4-Week Recovery Period in the Beagle Dog

8447423: Oral Gavage Embryo-Fetal Development Study Including Toxicokinetics with Lotilaner in Rabbits

(b) (4)*/TOX-005: Ocular; single rising dose toxicity; Beagle dog*

(b) (4)*/TOX-004: Ocular; 28-day toxicity; Dutch Belted rabbits*

(b) (4)*/ TOX-007: Ocular; 6-week toxicity; Dutch Belted rabbits*

(b) (4)*/TOX-006: Ocular; 7-day toxicity; Beagle dog*

(b) (4)*/ TOX-008: Ocular; 6-week toxicity; Beagle dog*

TRX-20-01: hERG assay in transfected HEK 293 cells

1275RT68.001: Oral, respiratory evaluation; Han Wistar rats

(b) (4)*10-076: Oral; single rising dose toxicity; Beagle dog*

(b) (4)*-12-001: Oral, 2-phase study, Han Wistar rats*

(b) (4)*-11-004: Oral; 14-day toxicity; Han Wistar rat*

(b) (4)*-11-018: Oral; 28-day toxicity; Han Wistar rat*

(b) (4)*-12-041: Oral; 13-week toxicity; Han Wistar rat*

(b) (4)*-11-019: Oral; 3-month toxicity; Beagle dog*

(b) (4)*-13-010: Oral; 8-month toxicity; Beagle dog*

(b) (4)*-12-042: In vitro bacterial reverse mutation assay*

(b) (4)-12-043: *In Vitro Induction of Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes*
(b) (4)12-044: *In Vivo Induction of Micronuclei in the Bone Marrow of Treated Rats*
12-036: Oral; Embryo-fetal development study; Han Wistar rat
13-008: Oral; 2-generation reproductive toxicity; Han Wistar rat
12-038: Single-Dose Ocular Irritation in Rabbits
13-006: In silico (Derek Nexus) Evaluation for Respiratory or Skin Sensitization
12-040: In Vitro Dermal Toxicity Evaluation (EpiDerm™)
12-037: Acute dermal toxicity in the rat
12-039: Murine local lymph node assay

3.2 Studies Not Reviewed

All "Methods and Validations" studies: 8420-804; 8420-805: PK; method validation Rabbit (-804) and dog (-805)

(b) (4)-14-053: *PK/ADME; oral and IV; single dose; Beagle dog*
(b) (4)-14-215: *PK; oral fed and unfed; single dose; Beagle dog*
(b) (4)-14-054: *PK/ADME; multiple doses; Han Wistar rat*
(b) (4)-11-017: *Oral; Embryo-fetal development study; dose-range finding study; Han Wistar rat*
(b) (4)-11-016: *Oral; 2-generation reproductive toxicity; dose-range finding; Han Wistar rat*
8448-932: Oral Gavage Pharmacokinetics and Pharmacodynamics Study with Lotilaner in Non- Pregnant Rabbits
(b) (4)-14-218: *Pivotal pharmacokinetic study of AHC°2224920 in cats following intravenous and oral administration*
(b) (4)-12-024: *Effect of feeding on the pharmacokinetics of AHC-2224920 after oral administration to dogs*
8445534: Evaluation of Lotilaner as a Substrate and/or Inhibitor of a Panel of Human Drug Transporters
8445532: Evaluation of Cytochrome P450 Induction Following Exposure of Primary Cultures of Human Hepatocytes to Lotilaner
8445533: Inhibitory Potential of Lotilaner towards Human Liver Microsomal Cytochrome P450 Enzymes
8454480: 14 Day Oral Gavage Dose Range Finding Study in Toxicity and Toxicokinetic Study in Mice (CByB6F1- Tg[HRAS]2Jic: Wild Type) with Lotilaner
8454485: 28 Day Oral Gavage Dose Range Finding Study in Toxicity and Toxicokinetic Study in Mice (CByB6F1- Tg[HRAS]2Jic: Wild Type) with Lotilaner

[redacted]: *A non-GLP 28 days repeat ocular dosing toxicology study in Dutch belted rabbits*

[redacted]: *Seven-day dose study of lotilaner by topical ocular administration to beagle dogs (non-GLP)*

8454872: *26-Week Oral Gavage Toxicity and Toxicokinetic Study in Rats with a 4-Week Recovery*

[redacted]*-14-053:* *PK/ADME; oral and IV; single dose; Beagle dog*

[redacted]*-14-215:* *PK; oral fed and unfed; single dose; Beagle dog*

[redacted]*-14-054:* *PK/ADME; multiple doses; Han Wistar rat*

[redacted]*-11-017:* *Oral; Embryo-fetal development study; dose-range finding study; Han Wistar rat*

[redacted]*-11-016:* *Oral; 2-generation reproductive toxicity; dose-range finding; Han Wistar rat*

8447422: *Oral Gavage Dose Range Finding Study Including Toxicokinetics with Lotilaner in Rabbits*

8447424: *Oral Gavage Tolerability Study with Lotilaner in Non-Pregnant Female Rabbits*

[redacted]*-11-017:* *AHC2224920: Oral (Gavage) Prenatal Developmental Range Finding Toxicity Study in the Rat*

[redacted]*-11-016:* *AHC2224920: Oral (Gavage) Range-Finding Study of General Reproductive Performance in the Rat*

3.3 Previous Reviews Referenced

For completeness, the reader is invited to refer to the review of IND143686 archived in DARRTS on 08-18-20[12].

IND [redacted]: Lotilaner; for the treatment of Lyme disease (Division of Anti-Infectives) (See below in the “Carcinogenicity Section” of this report”).

[12]https://darrts.fda.gov/darrts/faces/ViewDocument?documentId=090140af8058a15a

4 Pharmacology

4.1 Primary Pharmacology

Mechanism of action

Lotilaner is a member of the isoxazoline family of compounds. Isoxazolines, including Lotilaner, are selective inhibitors of insect and acarine GABA mediated chloride channels. Lotilaner is not an inhibitor of mammalian GABA mediated chloride channels when tested at up to 30 µM (18 µg/mL) *in vitro.*

The GABA-mediated chloride influx leads to hyperpolarization of the cellular membrane and generates an inhibitory postsynaptic potential, which decreases the probability of an action potential (Weber 2016). Ectoparasites exposed to isoxazolines will exhibit a spastic paralysis. In the case of *Demodex* mites, this paralysis leads to starvation and, ultimately, death.

Lotilaner has been shown to be effective against *Demodex* mites in dogs (Snyder 2017) and has been developed and approved as a veterinary drug for the eradication of skin parasites such as ticks and fleas.

Blepharitis in humans is commonly associated with infestation of eyelash follicles and meibomian glands by microscopic obligate parasitic mites, i.e., *Demodex folliculorum* or *Demodex brevis*, respectively. Currently, there are no approved pharmaceutical treatments for *Demodex* blepharitis.

Proof of concept studies were not conducted for this product.

4.2 Secondary Pharmacology

(b) (4) *095-0018934: In Vitro Pharmacology Study of Lotilaner*

Methods

Lotilaner was evaluated *in vitro* for off-target activity at the concentration of 10 µM (6 µg/mL) against a comprehensive panel of enzymes, ion channels, and receptors.

Results

Assays showing greater than 50% activity were potential CNS targets, including the melatonin receptor 1 (MT1), tachykinin receptor 2 (NK2), mu-opioid receptor (MOP), serotonin receptors (5-HT2A, 5-HT2B, and 5-HT6), GABA-gated chloride channel, sigma receptor, and the norepinephrine and dopamine transporters (Table 2).

Table 2: Lotilaner Binding Activity

Assay	Binding or Inhibition at 10 µM (%)
A_3 (h) (agonist radioligand)	96.2
α_{2c} (h) (antagonist radioligand)	64.3
MT_1 (h) (agonist radioligand)	67.8
NK_2 (h) (agonist radioligand)	53.9
µ (MOP) (h) (agonist radioligand)	55.3
FP (h) (agonist radioligand)	61.6
5-HT_{2A} (h) (agonist radioligand)	58.5
5-HT_{2B} (h) (agonist radioligand)	74.4
5-HT_{2C} (h) (agonist radioligand)	59.9
Sigma (non-selective) (h) (agonist radioligand)	73.0
GR (h) (agonist radioligand)	52.0
Ca^{2+} channel (L, dihydropyridine site) (antagonist radioligand)	92.8
Na^+ channel (site 2) (antagonist radioligand)	51.8
Cl^- channel (GABA-gated) (antagonist radioligand)	92.8
Norepinephrine transporter (h) (antagonist radioligand)	77.1
Dopamine transporter (h) (antagonist radioligand)	79.1
COX2 (h)	53.7

(Copied from (b)(4) 095-0018934 on p 5)

Functional implications of altering one or more of these protein functions may include psychoactivity and abuse liability (e.g., MOP, dopamine transporters, sigma receptor, and 5-HT), seizure liability (e.g., GABA-chloride channels), sympathetic activity, circadian rhythm, and sleep (e.g., norepinephrine and MT1).

> **This reviewer's comment:** As the functional potency (IC_{50}) of Lotilaner at these targets was not evaluated, it is not possible to predict an agonist or antagonistic effect *in vivo*. However, based on a predicted unbound fraction in the blood of less than 0.1% (See PK/ADME section) and the total C_{max} on the order of 0.2 to 0.4 µg/mL (*versus* 6 µg/mL, or 15X to 30X less than the concentration tested in secondary pharmacology) achieved at the NOAELs in 6-month rabbits and 9-month dogs ocular toxicity studies (see "Toxicology Section"), this reviewer considers the risk not significant for CNS-related side effects in the clinic, notwithstanding the fact that the test article would have to be able to cross the blood brain barrier. In addition, at the NOAELs, clinical systemic safety margins with the intended therapeutic dose were (b)(4) X and (b)(4) X with the dog and the rabbit studies, respectively, which diminished the risk even further.

4.3 Safety Pharmacology

From the review of IND143686[12]:
Safety pharmacology assessments of Lotilaner have been performed *in vitro* and *in vivo* in rats and dogs:

- Lotilaner did not induce any significant block of hERG current at concentrations up to 100 µM (60 µg/mL).
- In an 8-month oral GLP toxicity study in dogs, no Lotilaner-related effects on ECG parameters were observed at any time point over the duration of the study when animals received doses of Lotilaner at 43, 129, or 215 mg/kg, administered every 4 weeks. No Lotilaner-related effects on neurological endpoints were observed either.
- In a 6-week ocular GLP toxicity study in dogs, no Lotilaner-related effects on ECG parameters or heart rate were observed at 6 hours post the first daily dose (T_{max}) on Day 41 of the dosing phase when animals received Lotilaner Ophthalmic Solution, 0.25% at (b) (4) µg/animal/day. No effects on the respiratory rate were found.
- In a 13-week oral GLP toxicity study in rats, no Lotilaner-related neurobehavioral effects or effects on locomotor activity were observed over the duration of the study when animals were administered Lotilaner at 0, 5, 20, or 60 →40 mg/kg/day.
- In a GLP safety pharmacology study in male rats, Lotilaner was administered by oral gavage as a single dose at 20, 60 or 200 mg/kg. No Lotilaner-related effects on respiratory parameters (i.e., respiratory rate, tidal volume, or minute volume) were observed at any dose.

Based on the large safety margins (>1000X) achieved in these studies compared to the systemic therapeutic dose in mg/m^2 equivalent, the risk for humans was considered not significant by this reviewer.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

From the review of IND143686[12]:

ABSORPTION
Single dose administration
Oral and intravenous
From the review of IND143686[12]:

The absorption of AHC 2224920 was assessed after oral and intravenous administration in the dog. AHC 2224920 was absorbed and detected in plasma with a T_{max} between 2 and 4 hours. When fed dogs were dosed orally at 20 mg/kg, the $T_{1/2}$ was around 30 days. In fed versus fasted dogs, food significantly enhanced the bioavailability of AHC 2224920 (82% versus 24%, respectively). In another dog study, [^{14}C]-AHC 2224920 was given at 20 mg/kg orally or 5 mg/kg by IV injection. Oral bioavailability in this study was about 84%.

Ocular

The ocular absorption and distribution of Lotilaner, Ophthalmic Solution, 0.25% was assessed in a single-dose pharmacokinetics study in rabbits by ocular topical application. This study was not included the original IND143686[12] and will be reviewed herein.

21-TAR-002: SINGLE DOSE OCULAR PHARMACOKINETICS OF A 0.25% LOTILANER TOPICAL OPHTHALMIC FORMULATION TP-03 IN THE DUTCH BELTED (DB) RABBIT

Methods

Eighteen male DB rabbits received a single drop ((b) (4) mg/eye in (b) (4) µL) Lotilaner, Ophthalmic Solution, 0.25%/OU on Day 0. Whole blood samples were collected at 0.25, 2, 6, 24, 48, 72, 120, 168, and 336-hours post-dose in all surviving animals. Ocular tissue and fluid samples were collected from 2 animals immediately post euthanasia at each post-dose time point.

Results

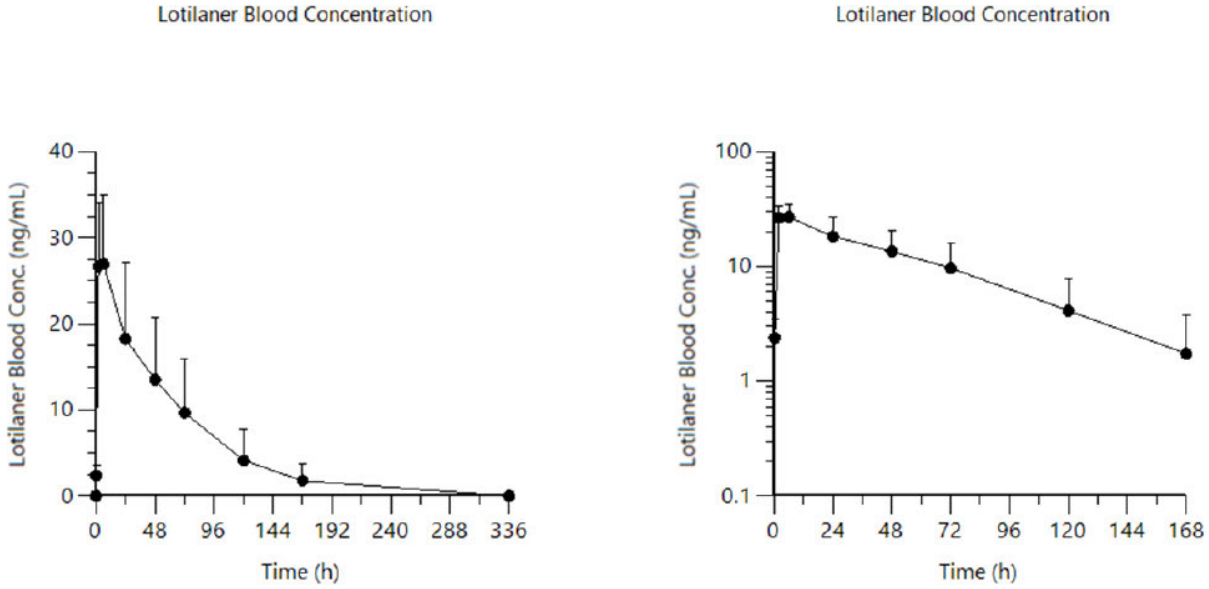
In the blood, Lotilaner was detected at the earliest time point of 0.25 h with T_{max} of 4-6 h, mean C_{max} of 27 ng/mL, and mean $T_{1/2}$ of 53.0 - 59.1 h (i.e., over 2 days) (Table 3, Figure 1). AUC_{last} was 1900 h.ng/mL and the apparent volume of distribution (Vz/F) of 6030 mL/h indicated large drug tissue distribution (rabbit blood volume about 60 mL/kg) (Table 4).

Table 3: Mean and Median Lotilaner Concentration in Blood

	Time (h)									
	0.00	0.250	2.00	6.00	24.0	48.0	72.0	120	168	336
	Blood Concentration (ng/mL)									
N	18	18	16	14	12	10	8	6	4	2
Mean	0.00	2.39	26.7	26.9	18.2	13.5	9.66	4.13	1.74	0.00
Median	0.00	2.01	25.9	28.6	19.9	14.9	10.1	4.35	1.50	0.00

(Copied from Study 21-TAR-002 on p 11)

Figure 1: Concentration Time Profile of Lotilaner Mean + SD (Linear and Log-Linear) in Blood Following Topical 0.25% Administration



(Copied from Study 21-TAR-002 on p 13)

Table 4: Lotilaner Blood Sparse Sampling PK Parameters

T_{max} (h)	T_{lag} (h)	C_{max} ± SE (ng/mL)	AUC_{last} ± SE (h*ng/mL)	AUC_{inf} (h*ng/mL)	% Extrap	V_z/F (mL)	CL/F (mL/h)	Rsq	$T_{1/2}$ (h)
6.0	0.00	26.9 ± 2.14	1900 ± 119	2190	13.5	6030	70.7	0.998	59.1

(Copied from Study 21-TAR-002 on p 18)

In the ocular tissues, Lotilaner exhibited a biphasic distribution and clearance with a mean terminal $T_{1/2}$ of 50-58 h (i.e., over 2 days) (Figure 2). Lotilaner distribution to the anterior chamber tissues, i.e., aqueous humor, iris-ciliary body was poor, or even nonexistent as in the lens (Table 5).

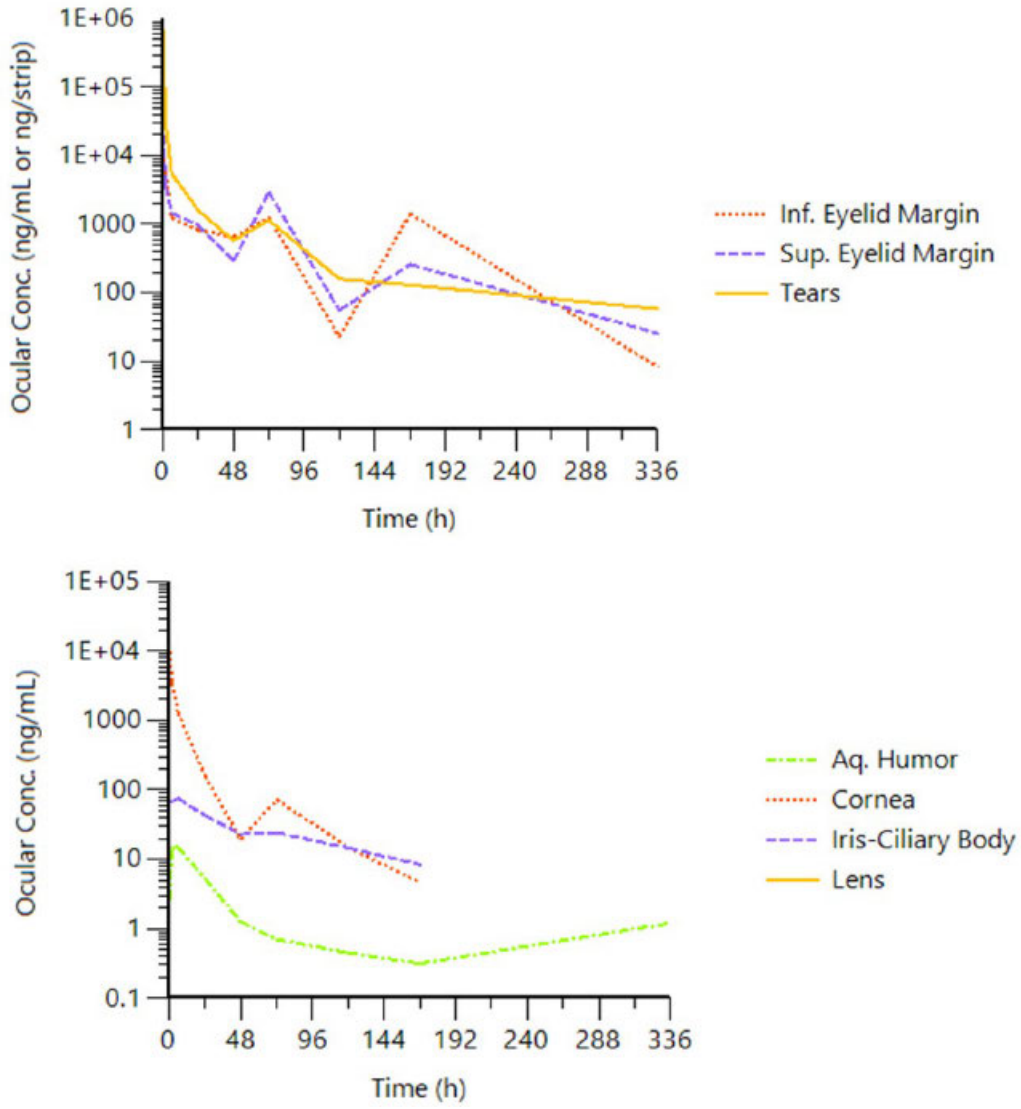
The clearance (CL/F), ranged from 3.49 mL/h to 7.89 mL/h for most ocular tissues except for the aqueous humor which had similar clearance to the blood compartment, i.e., about 70-90 mL/h and the iris/ciliary body where an intermediate clearance of 27.2 mL/h was observed. The tears, and the superior and inferior eyelid margins exhibited the slowest clearance, within the range of 0.567 mL/h to 0.720 mL/h.

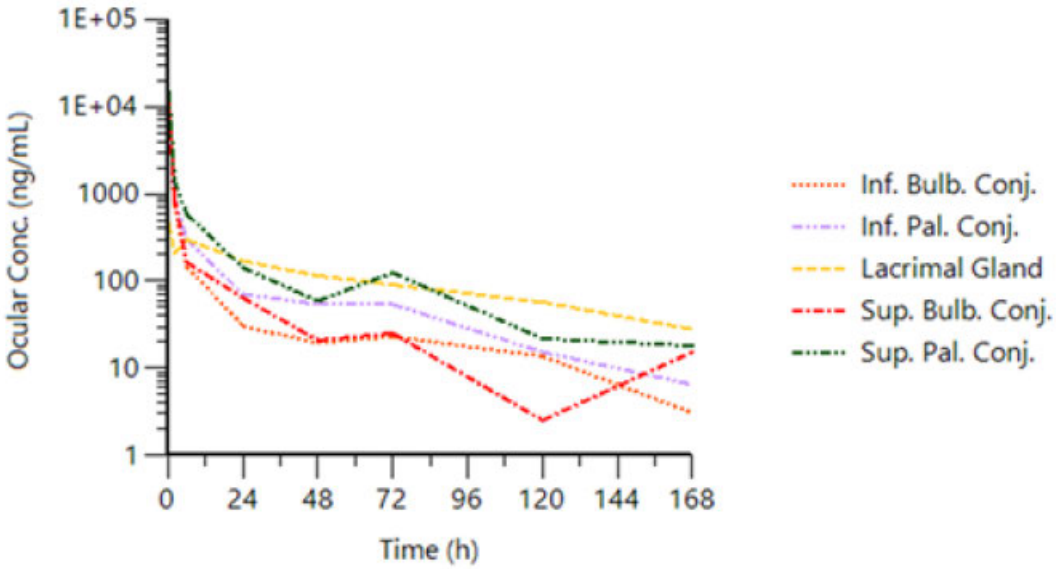
Mean C_{max} in the conjunctiva and inferior eyelid margin ranged from 10,000 ng/mL to 14,500 ng/mL, less than the average C_{max} in the superior eyelid margin of 20,100 ng/mL.

AUC_{inf} for the inferior and superior eyelid margins was 273,000 h*ng/mL and 215,000 h*ng/mL, respectively, i.e., 5- to 10-fold higher than for the other ocular tissues where it ranged from 44,400 (cornea) to 19,600 (lacrimal gland) (Table 6).

Overall, the data indicated that tears, inferior and superior eyelid margins (i.e., the drug targets) were prone to drug accumulation.

Figure 2: Mean Concentration-Time Profile of Lotilaner (Log-Linear) in Ocular Tissues Following Topical 0.25% Administration





(Copied from Study 21-TAR-002 on p 14-16)

Table 5: Mean and Median Lotilaner Concentration in Ocular Tissues

		Time (h)								
		0.250	2.00	6.00	24.0	48.0	72.0	120	168	336
Matrix		**Ocular Concentration (ng/mL)**								
AH	**Mean**	2.65	14.8	14.9	5.26	1.26	0.700	0.450	0.315	1.20
	Median	2.89	14.0	14.4	4.62	1.27	0.590	0.00	0.00	1.25
Cornea	**Mean**	9840	3480	1300	159	19.5	71.8	14.9	4.63	0.00
	Median	10000	2940	1290	95.9	22.1	29.5	11.3	0.00	0.00
IBC	**Mean**	11000	751	147	30.4	19.4	23.1	13.7	3.08	0.00
	Median	7930	681	136	29.0	20.4	18.1	14.6	0.00	0.00
ICB	**Mean**	0.00	67.7	75.4	42.7	23.0	24.3	14.5	8.40	0.00
	Median	0.00	72.2	74.7	40.2	23.2	22.8	14.6	10.5	0.00
IEM	**Mean**	9070	5530	1230	823	655	1250	22.7	1420	8.33
	Median	7920	2890	1150	627	568	760	14.3	11.5	7.65
IPC	**Mean**	13700	894	302	70.6	53.8	53.8	15.3	6.50	0.00
	Median	16200	886	189	56.4	50.6	51.2	15.2	0.00	0.00
Lens	**Mean**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Median	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LG	**Mean**	459	205	299	170	115	90.3	57.2	27.9	0.00
	Median	31.1	204	290	172	109	88.3	46.6	28.2	0.00
SBC	**Mean**	10800	892	163	63.8	20.9	25.0	2.53	15.3	0.00
	Median	9790	670	147	39.8	22.0	21.4	0.00	0.00	0.00
SEM	**Mean**	20100	3540	1450	972	288	2920	55.3	259	25.6
	Median	20800	2990	1150	902	313	1170	19.4	34.0	26.6
SPC	**Mean**	14500	1410	584	142	59.0	124	21.8	18.1	0.00
	Median	14500	1530	301	104	58.8	92.0	21.9	12.4	0.00
Tears*	**Mean**	682000	27600	5480	1600	579	1140	159	130	57.9
	Median	659000	4670	3030	1520	394	632	51.3	35.0	32.2

*Tears Units - ng/strip

AH - aqueous humor, **IBC** - inferior bulbar conjunctiva, **ICB** - iris-ciliary body, **IEM** - inferior eyelid margin, **IPC** - inferior palpebral conjunctiva, **LG** - lacrimal gland, **SBC** - superior bulbar conjunctiva, **SEM** - superior eyelid margin, **SPC** - superior palpebral conjunctiva

(Copied from Study 21-TAR-002 on p 17)

Table 6: Lotilaner Ocular Tissues Sparse Sampling PK Parameters

PK parameters	T_{max} (h)	T_{lag} (h)	C_{max} ± SE (ng/mL)	AUC_{last} ± SE (h*ng/mL)	AUC_{inf} (h*ng/mL)	% Extrap	V_z/F (mL)	CL/F (mL/h)	Rsq	$T_{½}$ (h)
Blood	6.0	0.00	26.9 ± 2.14	1900 ± 119	2190	13.5	6030	70.7	0.998	59.1
Tears[1]	0.25	0.00	682000± 381000	937000± 389000	949000	1.29	34.7	0.163	0.999	147
Cornea	0.25	0.00	9840±2930	43000±5050	44400	3.30	276	3.49	0.543	54.9
Aqueous Humor	6.0	0.00	14.9±3.72	757±56.3	1740	56.4	54500	89.3	0.118	423
Iris-Ciliary Body	6.0	0.250	75.4±10.2	4310±173	5700	24.4	3370	27.2	0.964	86.0
Inferior Bulbar Conjunctiva	0.25	0.00	11000±4240	17800±4310	20200	11.7	1470	7.67	0.825	133
Inferior Palpebral Conjunctiva	0.25	0.00	13700±4240	25700±4540	27600	6.88	410	5.62	0.683	50.6
Superior Bulbar Conjunctiva	0.25	0.00	10800±1710	19900±2070	24300	18.4	465	6.37	0.324	50.7
Superior Palpebral Conjunctiva	0.25	0.00	14500±4250	35500±6130	36800	3.73	240	4.21	0.751	39.5
Inferior Eyelid Margin	0.25	0.00	9070±3090	272000± 155000	273000	0.451	42.0	0.567	0.538	51.3
Superior Eyelid Margin	0.25	0.00	20100±6240	212000± 82700	215000	1.29	58.5	0.720	0.630	56.3
Lacrimal Gland	0.25	0.00	459±430	17300±871	19600	11.9	659	7.89	0.991	57.9
1- Tears concentration units were in ng/strip. Thus, AUC values are h*ng/strip										

(Copied from Study 21-TAR-002 on p 18) (copied from Study 21-TAR-002 on p 18)

This reviewer's comment: It would have been preferable for the applicant to study both genders.

Multiple dose administration

Oral

From the review of IND143686[12]:

The absorption of AHC 2224920 was assessed after oral administration in the rat and the dog.

- In a 13-week repeated dose GLP toxicity study in Wistar rat, the animals received orally by gavage AHC 2224920 once a day at 5, 20 or 60→40 mg/kg/day. T_{max} was 6 hours on Day 1 and 4-6 hours on Day 91. Exposure parameters (C_{max} and AUC_{0-24}) increased roughly proportionally to the dose on Days 1 and 91. Accumulation

was observed from Days 1 to 91, and no gender differences in exposure were observed.

- In an 8-month repeated dose GLP toxicity study in Beagle dogs, the animals received orally AHC 2224920 tablets at 43, 129 or 215 mg/kg every 4 weeks. The variability in C_{max} and AUC was low and similar between dose groups at each month, indicating that the dosage was continuous over the 8-month period. No differences in exposure were observed between genders. Mean systemic exposure ($AUC_{0-672hr}$) and C_{max} values of AHC 2224920 increased with increasing dose in a less than dose proportional manner. T_{max} was about 6 hours. $T_{1/2}$ values increased over time (Month 1 < Month 5 < Month 8) and did not change with increasing doses. $T_{1/2}$ values determined from the peak and trough values on Months 2, 3, 4, 6, and 7 showed the same trend and ranged from 202 to 210 hours (8.4 to 8.8 days), 237 to 263 hours (9.9 to 11.0 days), 258 to 316 hours (10.8 to 13.2 days), 544 to 681 hours (22.7 to 28.4 days), and 519 to 586 hours (21.6 to 24.4 days), respectively. Accumulation over time was assessed with $AUC_{0-672hr}$ values, which increased from Month 1 to Month 8 and was less than 2-fold. Mean accumulation ratios did not appear to change with increasing doses.

Ocular

The ocular absorption and distribution of Lotilaner Ophthalmic Solution, 0.25% was assessed in a repeated-dose pharmacokinetics in rabbit by ocular topical application. This study was not included in the original IND143686[12] and will be reviewed herein.

21-TAR-001: REPEAT DOSE OCULAR PHARMACOKINETICS OF A 0.25% LOTILANER TOPICAL OPHTALMIC FORMULATION TP-03 IN THE DB RABBIT

Methods

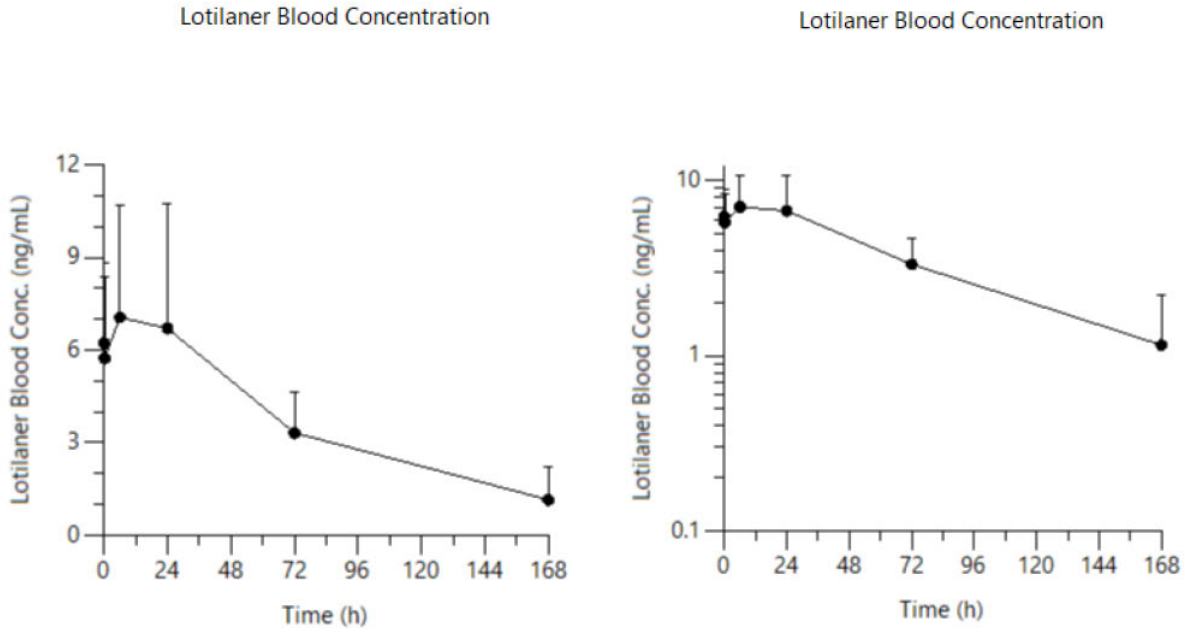
Sixteen males Dutch Belted (DB) rabbits were dosed twice daily with (b) (4) mg/eye/day/OU Lotilaner Ophthalmic Solution, 0.25% on Days 1-7 and once on Day 8. Whole blood samples were collected 6 h post the first of the two daily doses on Days 1 and 4. Whole blood was collected pre-dose on Day 8 and at 0.25, 6, 24, 72, 168, 240, and 336-hour post the final Day 8 dose. Terminal whole blood and ocular tissue samples were collected from 2 animals immediately post euthanasia at each time point post the Day 8 final dose.

Results

In the blood, Lotilaner accumulated up to 7-fold after multiple exposures. Mean C_{max} was observed at 6 h post-dose on each day and increased from 0.906 ng/mL on Day 1 to 7.06 ng/mL on Day 8 (Figure 3). On Day 8, systemic exposure decreased steadily through 10 days post last dose. Lotilaner mean blood terminal phase $T_{1/2}$ was 57.3 h (i.e., over 2 days) (Table 7). AUC_{last} was 617 h.ng/mL and the apparent volume of distribution (Vz/F)

of 18000 mL/h indicated large drug tissue distribution (rabbit blood volume is about 60 mL/kg) (Table 8).

Figure 3: Concentration Time Profile of Lotilaner Mean + SD (Linear and Log-Linear) in Blood Following Repeated Topical 0.25% Administration



(Copied from Study 21-TAR-001 on p 13)

Table 7: Lotilaner Blood Concentration

Day	1	4	8								
	Dose 1 +6h	Dose 1 + 6h	Pre-dose	0.25h	6h	24h	72h	168	240h	336h	672h
N	16	16	16	16	14	12	10	8	6	4	2
	Blood Concentration (ng/mL)										
Mean	0.903	5.84	6.22	5.74	7.06	6.70	3.32	1.15	0.00	0.00	0.00
SD	1.06	1.94	2.61	2.63	3.62	4.05	1.32	1.08	0.00	0.00	0.00
Median	0.510	5.59	5.62	5.72	6.46	5.78	3.46	1.13	0.00	0.00	0.00

(Copied from Study 21-TAR-001 on p11)

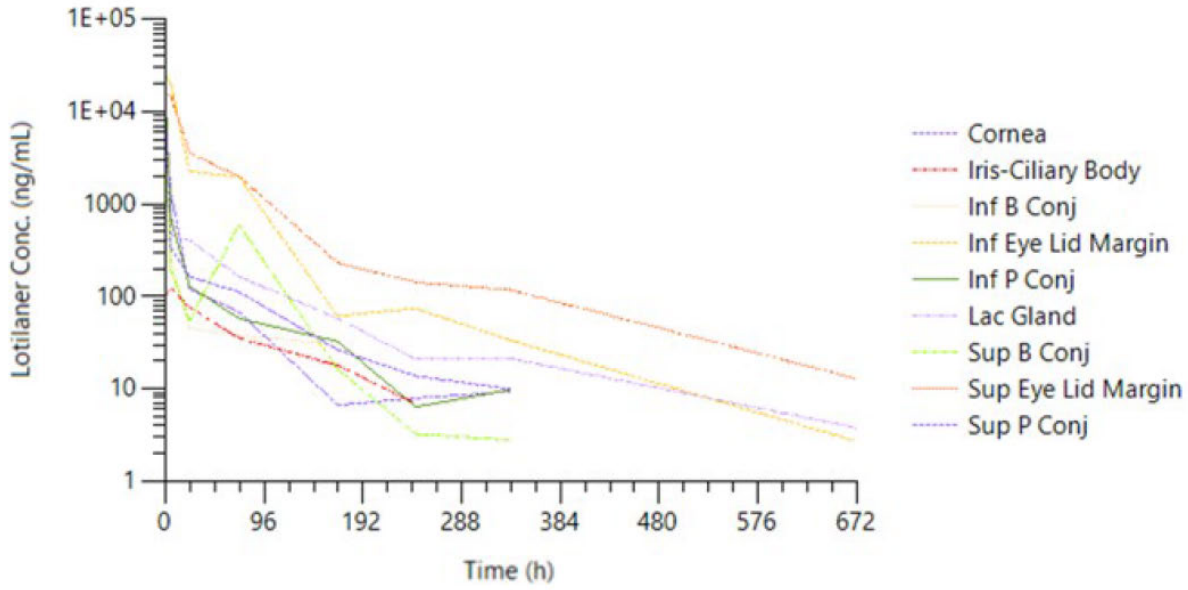
Table 8: Lotilaner Blood Sparse Sampling PK Parameters

T_{max} (h)	T_{lag} (h)	C_{max} ± SE (ng/mL)	AUC_{last}±SE (h*ng/mL)	AUC_{inf} (h*ng/mL)	%Extrap (%)	V_z/F (mL)	CL/F (mL/h)	Rsq	$T_{1/2}$ (h)
6.0	0.00	7.06±0.968	617±47.9	712	13.3	18000	218	0.995	57.3
SE=standard error									

(Copied from Study 21-TAR-001 on p 15)

In the ocular tissues, lotilaner mean terminal $T_{1/2}$ was higher than in the blood and in the range of 69.4 - 274 h (i.e., up to over 11 days) indicating accumulation in ocular tissues after multiple exposure compared to a single exposure (Table 9, figure 4). The average C_{max} was the highest in the eyelid margins and palpebral conjunctiva followed by the cornea, bulbar conjunctiva, lachrymal glands, and the iris/ciliary body. Overall, the data indicated that inferior and superior eyelid margins (drug targets) were prone to drug accumulation, coupled with the lowest clearance in these tissues (0.288 mL/h) and an AUC_{inf} 10-fold higher than in all other ocular tissues (Table 10).

Figure 4: Mean Concentration-Time Profile of Lotilaner (Log-Linear) in Ocular Tissues Following Repeated Topical 0.25% Administration



(Copied from Study 21-TAR-001 on p14)

Table 9: Mean and Median Lotilaner Concentration in Ocular Tissues

		Time Post Day 8 Dose (h)							
		0.250	6.00	24.0	72.0	168	244	336	672
Matrix		Ocular Concentration (ng/mL)							
IEM	Mean	24400	19400	2210	1920	60.3	72.8	32.9	2.65
	Median	23400	2650	2070	415	59.7	56.4	35.0	0.00
SEM	Mean	15800	14600	3490	1950	226	139	115	12.7
	Median	12400	6390	2660	1560	200	113	111	13.2
IPC	Mean	15300	684	126	56.6	31.9	6.23	9.58	0.00
	Median	16200	492	128	46.5	29.5	5.40	12.2	0.00
SPC	Mean	11700	326	159	110	25.8	13.4	9.73	0.00
	Median	12500	343	143	98.7	25.5	13.1	12.3	0.00
LG	Mean	376	453	402	160	56.9	20.4	20.9	3.73
	Median	359	464	421	160	57.6	18.1	26.0	0.00
Cornea	Mean	7270	1280	117	66.5	6.53	7.78	9.13	0.00
	Median	7330	1210	91.0	33.3	5.25	6.35	11.7	0.00
IBC	Mean	7830	247	43.8	37.1	29.3	0.00	5.58	0.00
	Median	7440	211	36.4	32.3	29.2	0.00	5.55	0.00
SBC	Mean	3780	186	53.7	589	16.0	3.18	2.73	0.00
	Median	3330	125	53.7	53.5	15.2	0.00	0.00	0.00
ICB	Mean	97.3	119	74.3	34.5	17.6	6.78	0.00	0.00
	Median	93.1	122	72.3	38.3	15.6	5.50	0.00	0.00

Inf. =inferior, **Sup.**=superior, **ELM**=eyelid margin, **Conj.**=conjunctiva, **B**=Bulbar, **P**=Palpebral, **ICB**=iris-ciliary body, **Lac**=lacrimal gland

(Copied from Study 21-TAR-001 on p15)

Table 10: Lotilaner Ocular Tissues Sparse Sampling PK Parameters
A: Exposure Parameters

Tissue	T_{max} (h)	C_{max} ± SE (ng/mL)	AUC_{last} ± SE (h*ng/mL)	AUC_{inf} (h*ng/mL)
Inf ELM	0.25	24400±3770	535000±234000	537000
Sup ELM	0.25	15800±5410	534000±136000	538000
Inf P Conj	0.25	15300±1380	66500±5320	68200
Sup P Conj	0.25	11700±3610	55900±11000	57200
Lac	6.0	453±24.2	46300±2590	52200
Cornea	0.25	7270±562	48600±5190	50300
Inf B Conj	0.25	7830±1090	35300±3490	37900
Sup B Conj	0.25	3780±612	60600±39200	62700
ICB	6.0	119±8.87	8660±622	10500

SE=standard error, **Inf.** =inferior, **Sup.**=superior, **ELM**=eyelid margin, **Conj.**=conjunctiva, **B**=Bulbar, **P**=Palpebral, **ICB**=iris-ciliary body, **Lac**=lacrimal gland

B: Clearance and Volume of Distribution

Tissue	V_z/F (mL)	CL/F (mL/h)	$T_{1/2}$ (h)	MRT_{inf} (h)
Inf ELM	76.3	0.288	183	40.3
Sup ELM	56.5	0.288	136	61.5
Inf P Conj	304	2.27	92.7	31.8
Sup P Conj	271	2.71	69.4	37.4
Lac	1170	2.97	273	236
Cornea	424	3.08	95.4	40.2
Inf B Conj	935	4.09	159	70.1
Sup B Conj	476	2.47	133	77.7
ICB	1990	14.8	93.1	125

Inf. =inferior, **Sup.**=superior, **ELM**=eyelid margin, **Conj.**=conjunctiva, **B**=Bulbar, **P**=Palpebral, **ICB**=iris-ciliary body, **Lac**=lacrimal gland

(Copied from Study 21-TAR-001 on p16)

This reviewer's comment: It would have been preferable for the applicant to study both genders.

DISTRIBUTION

From the review of IND143686[12]:

Lotilaner plasma protein binding at 5 µM (3 µg/mL) was high in mouse, rat, dog, and human, with unbound Lotilaner ≤ 0.1% in all species tested.

Tissue distribution of [^{14}C]-AHC 2224920 was evaluated in the Wistar rat and the Beagle dog after oral exposure. It was similar between both species with highest accumulation in liver > fat > muscle.

METABOLISM

In vitro

8445531: Metabolism of Lotilaner in Mouse, Rat, Rabbit, Dog, Monkey, and Human Primary Hepatocytes

Methods

Lotilaner (0.5 and 5 µM or 0.3 to 3 µg/mL) was incubated with mouse, rat, rabbit, dog, monkey, and human hepatocytes for 0, 30, 60, 90, 120, and 240 minutes. Lotilaner concentrations were determined by liquid chromatography mass spectrometry (LC-MS) and the amounts remaining from time zero (0) minutes were calculated.

Results
Lotilaner was metabolically stable in hepatocytes from all species in the conditions of this study. At 5 µM (3 µg/mL), the amount of Lotilaner remaining after incubation in hepatocytes for 4 h was 86.6 to 93.8% in the mouse, 80.2 to 95.0% in the rat, 95.4 to 100% in the rabbit, 88.0 to 100 in the dog, 93.3 to 100% in the monkey, and 96.3 to 100% in human. The changes in concentrations were not time dependent suggesting that no liver metabolism occurred (it was confirmed in human liver *in vitro* in Study 845547). Metabolite profiling was not conducted in any species due to the observed liver stability.

In vivo
From the review of IND143686[12]:
Metabolism was evaluated in the blood, urine, feces, and selected tissue samples (liver, kidney, fat, and muscle) in the Beagle dog and Wistar rat after oral administration. The metabolism in both species was similar. Negligible amounts of metabolites of the parent compound were identified in the blood, urine, and tissue samples with the parent compound being predominant. In the feces, several metabolite peaks were observed in addition to unmetabolized [^{14}C]-AHC 2224920. They had similar polarity to the parent compound or were slightly more polar. The most predominant of those being M.F3 in both species comprising 15-37% of the peak area and were believed to be the product of the biotransformation of the parent drug by opening of the isoxazoline ring followed by an oxidative deamination of the molecule.

EXCRETION
From the review of IND143686[12]:
The route and the extent of excretion of [^{14}C]-AHC 2224920 were studied in the Wistar rat following repeated oral gavage at 10 mg/kg/day for 6 days and in the Beagle dog after a single oral administration at 20 mg/kg. The excretion was quantified over 4 days after the last administration in the rat and at necropsy at Day 14 in the dog.

- In the rat, the primary excretion route was fecal. Fecal excretion was progressive, starting within the first 24 hours and after 4 days approximately 60% of the total administered dose was excreted (55.07% in feces, 3.74% in urine, and 0.73% in cage wash). Mean mass balance recovery was 93% at 48 hours post the last dose and 95% at 96 hours post the last dose.

- In the dog, the primary excretion route was also fecal. After oral administration, fecal excretion was progressive, starting within the first 24 hours; after 14 days, less than 50% of the administered dose was excreted (44.24% in feces, 2.07% in

urine, and 0.71% in cage wash). Mean mass balance recovery was 96.52% at 14 days after the last dose.

This reviewer's comments:

In a cat study ([(b) (4)]-14-055) by the oral route (data not reviewed), lotilaner was found to be excreted unchanged in the bile which allowed the drug product to be reabsorbed and to recirculate. This may explain the long $T_{1/2}$ and accumulation in tissues observed with this compound in all animal studies.

Based on the metabolic stability of lotilaner, this reviewer anticipated no disproportional or unique metabolites in humans *versus* the animal species used for preclinical development.

5.2 TK/Cross-Species Comparison

From the review of IND143686[12]:
The blood absorption of Lotilaner Ophthalmic Solution, 0.25%, by the ocular route of administration was assessed in 6-week GLP toxicity studies in Dutch Belted rabbits at the dose levels of [(b) (4)] µg/eye/day ([(b) (4)] µg/animal/day) and Beagle dogs at the dose levels of [(b) (4)] µg/eye/day ([(b) (4)] µg/animal/day).

The blood TK profile was largely similar between the dog and the rabbit. Steady state occurred between Days 21 and 42. Exposure (C_{max} and AUC_{0-24}) increased with the increase in dose level generally in a dose proportional fashion. The accumulation of Lotilaner was observed after multiple doses up to 7-fold in the rabbit and about 20 fold in the dog from Day 1 to day 42 and gender differences in Lotilaner exposure were less than 2-fold in both species.

The NOAEL in both studies was the highest dose tested of [(b) (4)] µg/eye/day ([(b) (4)] µg/animal/day). In the rabbit, this dose level corresponded to a mean peak concentration (C_{max}) and an area under the concentration-time curve (AUC_{0-24}) values of 177 ng/mL and 370 hr*ng/mL, respectively, in males and 126 ng/mL and 276 hr*ng/mL, respectively, in females on Day 42. In the dog, this dose level corresponded to mean peak concentration (C_{max}) and area under the concentration-time curve (AUC_{0-24}) values of 228 ng/mL and 499 hr*ng/mL, respectively, in males and 285 ng/mL and 605 hr*ng/mL, respectively, in females on Day 42.

The following chronic ocular toxicity studies in the DB rabbit and the Beagle dog were not reviewed with the Original IND143686[12]. They were reviewed herein and can be found in the "Repeat-Dose Toxicity" section of this report.

1020-7114: Lotilaner: A 6-Month Repeat-Dose Topical Ocular Toxicity Study Followed by a 4-Week Recovery Period in Dutch Belted Rabbits
1020-7122: Lotilaner: A 9-Month Repeat-Dose Topical Ocular Toxicity Study Followed by a 4-Week Recovery Period in the Beagle Dog

The blood absorption of Lotilaner Ophthalmic Solution, 0.25%, by the ocular route of administration was also assessed in a 6-month GLP toxicity study in Dutch Belted rabbits at the dose levels of (b) (4) µg/eye/day ((b) (4) µg/animal/day) and in a 9-month GLP toxicity study in Beagle dogs at the dose levels of (b) (4) µg/eye/day (b) (4) µg/animal/day).

The blood TK profile was largely similar between the dog and the rabbit. Exposure (C_{max} and AUC_{0-24}) increased with the increase in dose level generally in a dose proportional fashion. The accumulation of Lotilaner was observed after multiple doses up to 7-fold from Day 1 to day 182 in the rabbit and up to 27-fold from Day 1 to Day 273 in the dog, and gender differences in Lotilaner exposure were less than 2-fold.

The NOAEL in both species was the highest dose tested of (b) (4) µg/eye/day ((b) (4) µg/animal/day). In the rabbit, this dose level corresponded to a mean peak concentration (C_{max}) and an area under the concentration-time curve (AUC_{0-24}) values of 265 ng/mL and 5,660 hr*ng/mL, respectively, in males, and 237 ng/mL and 5,110 hr*ng/mL, respectively, in females on Day 182. In the dog, this dose level corresponded to a mean peak concentration (C_{max}) and area under the concentration-time curve (AUC_{0-24}) values of 436 ng/mL and 8,290 hr*ng/mL, respectively, in males, and 369 ng/mL and 7,290 hr*ng/mL, respectively, in females on Day 273.

6 General Toxicology

6.1 Single-Dose Toxicity

From the review of IND143686[12]:

- A study in the dog indicated that no systemic or ocular effects were observed in Lotilaner Ophthalmic Solution, 0.25%-treated animals versus controls. The maximum tolerated dose in this study was the high dose (2 drops/eye/2 eyes, three times daily, drop volume of (b) (4) µL = (b) (4) µg/eye/day) as the dose chosen for the 6-

week pivotal ocular toxicity study equivalent to (b) (4) µg/animal/day.

- In an acute oral study in rats, there were no test article-related effects on mortality, clinical signs, body weights, or necropsy findings. The acute median lethal oral dose level of AHC 2224920 was found to exceed 2000 mg/kg.

- In a single rising dose oral toxicity study in dogs, the test article was well tolerated when administered every 2 weeks under fed conditions. Single episodes of diarrhea were noted within 24 hours post-dose in 2 and 4 animals at 90 and 150 mg/kg, respectively. A slight increase in creatinine and bilirubin (BUN) values were observed in 3 males at 150 mg/kg. Dogs had measurable levels of drug at 24- and 48-hours post-dose and increases in exposure were dose-proportional.

6.2 Repeat-Dose Toxicity

From the review of IND143686[12]:

- In 2 ocular GLP toxicity studies in the Dutch Belted rabbit and in the Beagle dog for 6 weeks, no test article-related findings were noted up to the highest dose tested of (b) (4) µg/eye/day Lotilaner Ophthalmic Solution, 0.25% ((b) (4) µg/eye/dose), administered 3 times daily approximately 4 hours apart for 6 weeks. Thus, the ocular NOAEL in rabbits and dogs was (b) (4) µg/eye/day while the systemic NOAEL were (b) (4) µg/kg and (b) (4) µg/kg, respectively, based on 1.8 kg rabbit and a 10 kg dog.

- In an oral gavage GLP toxicity study in Han Wistar rats with AHC 2224920 at 5, 20, and 60→40 mg/kg/day for 13 weeks consecutively, the NOAEL was 20 mg/kg/day for males and 40 mg/kg/day for females. At Day 91 C_{max} and $AUC_{0\text{-}24hr}$ values achieved for males and females combined were 56 µg/mL and 1101 µg*hr/mL, respectively, at 20 mg/kg/day and 97 µg/mL and 1879 µg*hr/mL, respectively, at 40 mg/kg/day. Above NOAELs, mortality occurred at doses ≥ 40 mg/kg/day in males and at 60 mg/kg/day in females. The cause of death/moribundity was uncertain and was characterized by marked body weight loss. Other findings in surviving animals were found in adrenal and spleen (reversible), pituitary, thymus, and ovary (partial reversibility), and lung (no reversibility).

- In an oral tablet GLP toxicity study in the Beagle dog with AHC 2224920 at 43, 129, and 215 mg/kg, once per month for 8 total doses, the test article was well tolerated, and the NOAEL was the highest dose tested. Mean Month 8 C_{max} and

$AUC_{0\text{-}672hr}$ achieved were 21.3 µg/mL and 9870 µg *hr/mL, respectively (males and female combined) (Tables 11, 12).

Table 11: Ocular Safety Margin Based on Ocular NOAEL in the Pivotal Toxicology Studies in Support of IND143686[12]

Species	NOAEL	Total Dose (mg/eye/day)	Margins of Exposure Based on Total Dose
			(b) (4) mg/eye/day (Highest Intended Clinical Dose)
Rabbit	0.25% 2 drops ((b) (4) µL drop) *per* dose/OU TID	(b) (4)	2.66
Dog	0.25% 2 drops ((b) (4) µL drop) *per* dose/OU TID	(b) (4)	2.66

(Copied from the Pharmacology and Toxicology review of IND143686[12] on p 12)

Table 12: Systemic Safety Margins Based on Systemic NOAELs in the Pivotal Toxicology Studies in Support of IND143686[12]

Nonclinical				Clinical Safety Margins (Based on Dose)
Species/ Duration	NOAEL (mg/kg/day)	HED (mg/kg/day)	[4]C_{max} µg/mL /AUC µg.h/mL	MRHD[2] ((b) (4) mg/kg/day)
Rabbit/6 weeks ocular instillations	(b) (4)		0.152/3.23	28.7
Rat/13 weeks oral gavage	20 (M); 40 (F)	3.226 (M); 6.452 (F)	[5]56/1101; [6]97/1879	(b) (4)
Dog/6 weeks ocular instillations	(b) (4)		0.257/5.52	8.9
Dog/8 months oral tablets	215	119.444	21.3/9870	(b) (4)

NOAEL = no-observed-adverse-effect level
AUC = area under the concentration-time curve
MRHD = maximum recommended human dose
[3] Calculation based on a 1.8 kg BW
[5] Based on NOAEL of 20 mg/kg/day
[7] Calculation based on a 10 kg BW
HED = human equivalent dose
C_{max} = maximal concentration
[2] Calculation based on a 60 kg BW adult
[4] Males (M) and females (F) combined
[6] Based on NOAEL of 40 mg/kg/day

(Copied from the Pharmacology and Toxicology review of IND143686[12] on p 13)

For the marketing application, two supplementary chronic ocular GLP toxicity studies in the rabbit and the dog were submitted by the Sponsor (not reviewed with IND143686[12]) and were reviewed herein.

6.2.1 Study Title: AUDITED FINAL REPORT Lotilaner: A 6-Month Repeat-Dose Topical Ocular Toxicity Study Followed by a 4-Week Recovery Period in Dutch Belted Rabbits

Study no.:	1020-7114
Study report location:	eCTD0001 Module 4.2.3.2
Study initiation date:	December 29, 2020
Conducting laboratory and location:	(b) (4)
Duration:	6 months
Duration Units:	months
GLP compliance:	Draft
Drug, lot #, and % purity:	Lotilaner Ophthalmic Solution 0.25%; Lots 182130 and 183139; purity 100.3-100.8% of label claim

Key results

The topical ocular instillation of Lotilaner Ophthalmic Solution, 0.25%, administered bilaterally (OU), twice (BID) or three times (TID) daily to Dutch Belted (DB) rabbits at (b) (4) for 6 months was well tolerated. The local, ocular, NOAEL was (b) (4) µg/eye/day, and the systemic NOAEL was (b) (4) µg/kg for a 1.8 kg DB rabbit. At the NOAEL, Day 182 AUC_{0-24} and C_{max} were 5660 and 5110 hr*ng/mL (for AUC_{0-24}), and 265 and 237 ng/mL (for C_{max}) for male and female rabbits, respectively.

Note that the histopathology of the nasal cavities could not be assessed in this study as the nasal turbinate and nasopharynx, important target organs of toxicity after topical, ocular administration, were not processed for microscopy.

Methods

Doses:	[redacted] µg/eye/day, 2 eyes (OU)
Frequency of dosing:	BID or TID
Number/Sex/Group:	4 or 6
Dose volume:	[redacted] µL drop volume
Formulation/Vehicle:	Vehicle for Lotilaner Ophthalmic Solution 0.25% (clinical formulation)
Route of administration:	TOPICAL OCULAR INSTILLATION
Species:	RABBIT
Strain:	DUTCH BELTED
Age / Sexual Maturity:	5.5 months/Yes
Comment on Study Design and Conduct:	See study design below. Animals in Groups 1, 3, and 4 were administered 2 drops/eye/dose; the second drop was administered immediately after visual confirmation of the first drop dispersion. Groups 1 and 4 were dosed TID (3X daily) 4 hours apart and Groups 2 and 3 were dosed BID (2X daily) 8 hours apart.
Dosing Solution Analysis:	NA (product was utilized as delivered)

Treatment Group	Dose level (µg/eye/day)	Dose level (µg/eye/dose)	Test Item Conc. (w:v)	Volume/eye/dose (µL) [2]	Dosing Regimen (drops/eye/dose) [2]	Dosing Frequency [3]	Number of Animals			
							Main		Recovery	
							M	F	M	F
1. Control [1]	0	0	0 %	[redacted]	2	TID	4	4	2	2
2. Lotilaner -Low Dose	[redacted]		0.25 %		1	BID	4	4	-	-
3. Lotilaner -Mid Dose					2	BID	4	4	2	2
4. Lotilaner -High Dose					2	TID	4	4	2	2

[1] Group 1 animals received the reference item, Lotilaner Ophthalmic Solution vehicle.
[2] Each drop dispensed a target of [redacted] µL, as per device provided by the Sponsor. Animals in Groups 1, 3, and 4 were administered two drops/eye/dose; the second drop was administered immediately after visual confirmation of the first drop dispersion.
[3] Groups 1 and 4 were dosed three times daily (TID) 4 hours apart (±15 minutes); Groups 2 and 3 were dosed twice daily (BID) 8 hours apart (±15 minutes).
M: male; F: female; Conc. Concentration.

Observations and Results

Mortality

There were no premature deaths during the study.

Clinical Signs

There were no Lotilaner-related clinical observations at any dose level.

Body Weights

There were no Lotilaner-related effects on body weights or body weight changes at any dose.

Feed Consumption

There were no Lotilaner-related effects on food consumption at any dose.

Gross Ocular Observation

An evaluation of eye reactions for hyperemia (redness, congestion), swelling (chemosis) and discharge was performed once during the pre-dose period, on Day 1 of dosing [2 hours ± 1 hour following the last daily dose (relative to the end of dosing of each group)], prior to the first dose on Day 2, then weekly during the first month of dosing and once every two weeks thereafter (prior to the first daily dose), up to the end of dosing period.

There were no Lotilaner-related gross ocular findings (redness, swelling or presence of discharge) at any dose.

Ophthalmology and Tonometry

Funduscopic (indirect ophthalmoscopy), biomicroscopic (slit lamp) examinations were performed on all animals once during the pre-dose period and once towards the end of Weeks 5, 8, 13 and 26. Intra-ocular pressure (IOP) measurements were performed on all animals, conscious, on the same occasion as ophthalmological examinations. All ophthalmological examinations and IOP measurements were conducted by a board-certified veterinary ophthalmologist.

There were no Lotilaner-related ophthalmological findings or effects on ocular pressure at any dose.

Electroretinography (ERG)

ERGs were recorded from all animals once during the pre-dose period, and towards the end of dosing period (Week 26/27).

Each ERG occasion was performed as *per* Study Specific Procedure (SSP) and consisted of the following series of stimuli:

Step	Log scale (dB)	Number to average	Time between flashes
Scotopic Phase – following dark adaptation			
1	-30	5	10 seconds
2	-10	5	15 seconds
3	0	2	Minimum 2 minutes
Photopic Phase – following adaption to background light (25 to 30 cd/m^2) for at least 5 minutes			
4	0	20	1 second (1 Hz)
5	0	20	0.034 seconds (29.4 Hz)

Baselines were corrected when necessary to correct the waveform drift. Waveforms were analyzed for a-wave, b-wave, and flicker amplitudes and implicit times/latency.

The individual post-treatment results of ERG and tonometry was baselined-adjusted (2-week pre-dose period) and only baseline adjusted data was submitted to statistical group comparison for a-wave, b-wave amplitudes, and flicker.

Group comparisons were performed with Dunnett's and Dunn's tests.

There were no apparent Lotilaner-related effects on scotopic or photopic a- or b-wave amplitudes or implicit times.

Clinical Pathology

Clinical pathology evaluations (hematology, clinical chemistry, and coagulation) were performed on all animals once during the pre-dose period and on Day 183 (were collected prior to necropsy for Main animals on Day 183).

Hematology and Coagulation

Parameters evaluated

Cell morphology*
Hematocrit
Hemoglobin
Mean corpuscular hemoglobin
Mean corpuscular volume
Mean corpuscular hemoglobin concentration
Platelet count
Red blood cell count
Red cell distribution width
Reticulocyte counts (absolute and relative)
White blood cell count (WBC)
WBC differential (absolute and relative)

** Manual reading of smears for morphology check only were carried out as necessary.*

Activated partial thromboplastin time
Prothrombin time
Fibrinogen
Sample appearance (normal)

There were no Lotilaner-related effects on hematology and coagulation parameters at any dose.

Clinical Chemistry
Parameters evaluated:

Sample Appearance (when abnormal)	Creatinine
A/G ratio (calculated)	Globulin (calculated)
Alanine aminotransferase	Glucose
Albumin	Phosphorus (inorganic)
Alkaline phosphatase	Potassium
Aspartate aminotransferase	Protein (total)
Bilirubin (total)	Sodium
Calcium (total	Triglycerides
Chloride	Urea
Cholesterol (total)	

There were no Lotilaner-related effects on chemistry parameters at any dose.

Urinalysis
NA

Gross Pathology
Performed on Day 183 (Main) or Day 211 (Recovery). Terminal body weights were recorded for all animals.

There were no local (ocular or extraocular structures) or systemic gross findings considered related to the topical ocular administration of Lotilaner in the Main and Recovery phases of this study.

Organ Weights
Organ weights, as indicated below, were recorded at each scheduled sacrifice. Paired organs were weighed together. Relative organ weights (relative to terminal body weight and to brain weight) were calculated and reported.

Adrenals	Liver	Spleen
Brain	Lungs (with bronchi)	Testes
Epididymides	Ovaries (without oviduct)	Thymus
Heart	Pituitary	Thyroids with parathyroids
Kidneys	Prostate	Uterus (with cervix)

There were no organ weight changes considered to be related to the administration of Lotilaner in the Main and Recovery phases of this study.

Histopathology

Tissues preserved:

Abnormal findings	Heart [3]	Sciatic nerve
Adrenals	Ileum	Seminal vesicles
Animal identification (microchip) [1]	Jejunum	Skeletal muscle (thigh)
Aorta (thoracic)	Kidneys	Skin, subcutis & mammary gland (inguinal) [7]
Brain [2]	Lacrimal glands	Spinal cord (thoracic)
Cecum	Liver (2 lobes)	Spleen
Colon	Lungs (2 lobes) with bronchi [4]	Sternum with marrow
Duodenum	Lymph nodes (mandibular and mesenteric)	Stomach
Epididymides	Nictitating membranes	Testes [6]
Esophagus	Optic nerves (additional portion)	Thymus
Eyes (including a portion of the optic nerve) [6]	Ovaries	Thyroids with parathyroids [8]
	Pancreas	Tongue
Eyelids (upper and lower)	Pituitary	Trachea
Extraocular muscles [9]	Prostate	Urinary bladder
Femur with marrow	Rectum	Uterus [5]
Gallbladder	Salivary gland (mandibular)	Vagina
Harderian glands		

1. Fixation and preservation only.
2. Cerebral cortex, midbrain, cerebellum and medulla. Trimmed as per *Bolon B. et al. (2013)*.
3. Section included both ventricles and atria, septum with papillary muscle.
4. Lungs were infused with formalin.
5. Horns, body and cervix.
6. Fixed in Davidson's solution
7. Mammary glands were examined histologically only when present in routine sections.
8. Parathyroids were examined histologically only when present in routine sections.
9. Rectus dorsalis, rectus medialis, rectus ventralis, and rectus lateralis.

Bone marrow smears were prepared but not evaluated.

All tissues in the table were examined under the microscope for Main and Recovery animals of Groups 1 (Control) and 4 (High Dose), and any gross abnormalities from animals of all groups. Detailed ocular histopathology were performed from both eyes of animals selected for histopathological examination: eye globes, optic nerves (longitudinal and transverse sections), extraocular muscles, eyelids (upper and lower), harderian and lacrimal glands, and nictitating membranes. Five sagittal sections of each eye were prepared, with at least two sections containing the optic nerve and one section taken through the equivalent of the macula/fovea (visual streak).

Adequate Battery: Nasal turbinate and nasopharynx are missing from this list. These are important organs to observe under the microscope since the test compound accumulated in tears.

Peer Review: No

There were no local (ocular or extraocular structures) or systemic microscopic changes considered to be related to the topical ocular administration of lotilaner in the Main and Recovery phases of this study.

Special Evaluation
NA

Toxicokinetics
Blood samples were collected from all animals at the following targeted time points relative to the Days 1 and 182 dose administrations (prior to any subsequent dosing): Pre-dose, and at 1, 5, 9, 12, 24 hours post dose (relative to the first daily dose administration). Additionally, a blood sample was collected from all Recovery animals on Day 211 (prior to necropsy).

On Day 1, lotilaner blood concentrations increased slowly to peak between 9.00 to 12.0 hr (after the second or third daily administrations) and stayed above BQL up to 24 hr sampling.

On Day 182, lotilaner blood concentrations were plateaued up to 24 hr post first daily dose. On both Day 1 and Day 182, the rate and extent of exposures (C_{max} and AUC_{0-t}) increased in a dose proportional manner as the dose increased in females and males, respectively.

Following repeated dosing, accumulation (5-7-fold) was observed for all dose levels and sex. No notable sex difference was observed.

This resulted in Day 182 female mean C_{max} for the (b) (4) µg/eye/day lotilaner dose groups of 110, 173 and 237 ng/mL, while mean AUC_{0-24} were 2280, 3680, and 5110 h*ng/mL, respectively. For males on Day 182, mean C_{max} for the (b) (4) µg/eye/day lotilaner dose groups were 93.1, 157 and 265 ng/mL, while mean AUC_{0-24} were 2000, 3400, and 5660 h*ng/mL, respectively (Table 13).

Table 13: Summary of TK parameters
DAY 1

			C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (h*ng/mL)	AUC_{0-24} (h*ng/mL)	DN C_{max} (ng/mL/ug)	DN AUC_{0-24} (h*ng/mL/ug)
Day	Treatment	Sex	Mean (CV%); n[a]					
1	(b) (4) µg/eye/day Lotilaner (BID)	F	17.1 (20.6); 4	12.0 (12.0-12.0); 4	339 (14.9); 4	339 (14.9); 4	0.0551 (20.6); 4	1.09 (14.9); 4
	(b) (4) µg/eye/day Lotilaner (BID)	M	17.5 (13.6); 4	10.5 (9.00-12.0); 4	343 (13.2); 4	343 (13.2); 4	0.0565 (13.6); 4	1.11 (13.2); 4
	(b) (4) µg/eye/day Lotilaner (BID)	F	30.0 (22.6); 6	12.0 (9.00-12.0); 6	568 (22.9); 6	568 (22.9); 6	0.0484 (22.6); 6	0.916 (22.9); 6
	(b) (4) µg/eye/day Lotilaner (BID)	M	25.4 (36.9); 6	12.0 (9.00-12.0); 6	466 (43.1); 6	464 (42.9); 6	0.0410 (36.9); 6	0.748 (42.9); 6
	(b) (4) µg/eye/day Lotilaner (TID)	F	42.6 (32.5); 6	10.5 (1.00-12.0); 6	813 (36.4); 6	813 (36.4); 6	0.0458 (32.5); 6	0.875 (36.4); 6
	(b) (4) µg/eye/day Lotilaner (TID)	M	48.1 (28.4); 6	10.5 (9.00-12.0); 6	919 (34.4); 6	919 (34.4); 6	0.0517 (28.4); 6	0.988 (34.4); 6

(Copied from Study 1020-7114 on p 33)

DAY 182

			C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (h*ng/mL)	AUC_{0-24} (h*ng/mL)	DN C_{max} (ng/mL/ug)	DN AUC_{0-24} (h*ng/mL/ug)
Day	Treatment	Sex	Mean (CV%); n[a]					
182	(b) (4) µg/eye/day Lotilaner (BID)	F	110 (15.1); 4	24.0 (24.0-24.0); 4	2280 (8.8); 4	2280 (8.8); 4	0.354 (15.1); 4	7.34 (8.8); 4
	(b) (4) µg/eye/day Lotilaner (BID)	M	93.1 (13.2); 4	18.0 (12.0-24.0); 4	2000 (9.4); 4	2000 (9.4); 4	0.300 (13.2); 4	6.45 (9.4); 4
	(b) (4) µg/eye/day Lotilaner (BID)	F	173 (21.2); 6	18.0 (5.00-24.0); 6	3470 (9.7); 5	3680 (16.4); 6	0.280 (21.2); 6	5.94 (16.4); 6
	(b) (4) µg/eye/day Lotilaner (BID)	M	157 (41.0); 6	12.0 (12.0-24.0); 6	3210 (48.4); 5	3400 (43.1); 6	0.253 (41.0); 6	5.49 (43.1); 6
	(b) (4) µg/eye/day Lotilaner (TID)	F	237 (22.5); 6	18.0 (0.00-24.0); 6	5150 (21.9); 5	5110 (19.9); 6	0.255 (22.5); 6	5.49 (19.9); 6
	(b) (4) µg/eye/day Lotilaner (TID)	M	265 (23.9); 6	24.0 (5.00-24.0); 6	5760 (26.2); 5	5660 (24.3); 6	0.285 (23.9); 6	6.08 (24.3); 6

[a] T_{max} is presented with Median (Min-Max); n
F = females; M = males; n = number of observations

(Copied from Study 1020-7114 on p 34)

SEX RATIOS

Day	Treatment	Sex Comparison	Ratio C_{max}	Ratio AUC_{0-24}
1	(b) (4) µg/eye/day Lotilaner (BID)	Male/Female	1.02	1.01
	µg/eye/day Lotilaner (BID)	Male/Female	0.846	0.817
	µg/eye/day Lotilaner (TID)	Male/Female	1.13	1.13
182	µg/eye/day Lotilaner (BID)	Male/Female	0.848	0.879
	µg/eye/day Lotilaner (BID)	Male/Female	0.905	0.925
	µg/eye/day Lotilaner (TID)	Male/Female	1.12	1.11

(Copied from Study 1020-7114 on p 34)

DOSE RATIOS

Day	Sex	Dose Comparison	Dose Ratio	Ratio C_{max}	Ratio AUC_{0-24}
1	Female	(b) (4)	2.00	1.76	1.68
	Female		3.00	2.49	2.40
	Female		1.50	1.42	1.43
	Male		2.00	1.45	1.35
	Male		3.00	2.75	2.68
	Male		1.50	1.89	1.98
182	Female		2.00	1.58	1.62
	Female		3.00	2.16	2.25
	Female		1.50	1.37	1.39
	Male		2.00	1.68	1.70
	Male		3.00	2.84	2.83
	Male		1.50	1.69	1.66

(Copied from Study 1020-7114 on p 34)

This reviewer's comment: Note that $T_{1/2}$ was not reported in this study due to "an inability to characterize the elimination phase" (*per* the Sponsor). Preliminary clinical PK study results in healthy volunteers indicated a $T_{1/2}$ of 1400 hours or 58 days at Day 42 after repeated ocular administration (personal communication with Dr. Amit Somani, clinical pharmacologist in CDER). Hence, this reviewer suspects that the 4-week recovery period in this study was not 5 half-lives as recommended for the length of the recovery period. However, this was acceptable since no toxicity was found.

6.2.2 Study Title: AUDITED FINAL REPORT 9-Month Repeat-Dose Topical Ocular Toxicity Study in Dogs Followed by a 4-Week Recovery Period

Study no.:	1020-7122
Study report location:	eCTD0001 Module 4.2.3.2
Study initiation date:	January 6, 2021
Conducting laboratory and location:	(b) (4)
Duration:	9 months
Duration Units:	months
GLP compliance:	Y
Drug, lot #, and % purity:	Lotilaner Ophthalmic Solution 0.25%; Lots 182130 and 183139, purity 100.3-100.8% of label claim

Key results

No test article-related findings were noted following the administration of up to (b) (4) µg/eye/day ((b) (4) µg/eye/dose three times daily approximately 4 hours apart) Lotilaner Ophthalmic Solution, 0.25%, *via* topical ocular instillation to male and female Beagle dogs for 9 months. Thus, the local, ocular NOAEL was (b) (4) µg/eye/day, and the systemic NOAEL was (b) (4) µg/kg for a 10 kg beagle dog. This dose level corresponded to an AUC_{0-24} and C_{max} of 8290 and 7290 h*ng/mL (AUC_{0-24}), and 436 and 369 ng/mL (C_{max}) for male and female beagle dogs, respectively, on Day 273 of the dosing phase.

Note that the histopathology of the nasal cavities could not be assessed in this study as the nasal turbinate and nasopharynx, important target organs of toxicity after topical, ocular administration, were not processed for microscopy.

Methods

Doses:	[redacted] µg/eye/day, 2 eyes (OU)
Frequency of dosing:	BID or TID
Number/Sex/Group:	2 or 4
Dose volume:	[redacted] µL drop volume
Formulation/Vehicle:	Vehicle for Lotilaner Ophthalmic Solution 0.25%
Route of administration:	TOPICAL OCULAR INSTILLATION
Species:	DOG
Strain:	BEAGLE
Age / Sexual Maturity:	6.4 to 7.3 months old/No
Comment on Study Design and Conduct:	See study design below. Animals in Groups 1, 3, and 4 were administered 2 drops/eye/dose; the second drop was administered immediately after visual confirmation of the first drop dispersion. Groups 1 and 4 were dosed TID (3X daily) 4 hours apart and Groups 2 and 3 were dosed BID (2X daily) 8 hours apart.
Dosing Solution Analysis:	NA (product was used as delivered)

Treatment Group	Test Item Concentration (%)	Dose level (µg/eye/day)	Dose level (µg/eye/dose)	Volume/eye/dose (µL) [2]	Dosing Regimen (drops/eye/dose)[2]	Daily Dosing Frequency[3]	Number of Animals			
							Main		Recovery	
							M	F	M	F
1. Control [1]	0	[redacted]			2	TID	4	4	2	2
2. Lotilaner - Low Dose	0.25				1	BID	4	4	-	-
3. Lotilaner - Mid Dose					2	BID	4	4	-	-
4. Lotilaner - High Dose					2	TID	4	4	2	2

[1] Group 1 animals received the reference item.
[2] Each drop dispensed a target of [redacted] µL, as per device provided by the Sponsor. Animals in Groups 1, 3, and 4 were administered two drops/eye/dose; the second drop was administered after visual confirmation of the first drop dispersion.
[3] Groups 1 and 4 were dosed three times daily (TID), 4 hours apart (± 15 minutes); Groups 2 and 3 were dosed twice daily (BID), 8 hours apart (± 15 minutes).
M: male; F: female.

Observations and Results

Mortality

No test article related mortalities occurred in this study.

Male animal No. 4003, given (b) (4) µg/eye/day of Lotilaner, was euthanized on Day 37 presenting severe weakness, and deteriorating condition due to an intestinal intussusception which correlated microscopically to necrosis and hemorrhages of the ileal wall. Intussusceptions can occasionally occur spontaneously in dogs. It was interpreted as an incidental spontaneous event, and not related to the administration of Lotilaner. This reviewer agreed with this assessment as it was a single, isolated incident occurring in one animal among all dogs used in 2 different GLP toxicity studies at this dose of Lotilaner. Furthermore, no intestinal toxicity was found in the chronic oral studies with the dog at much higher doses of Lotilaner.

Clinical Signs

No Lotilaner-related clinical observations occurred at any dose.

Body Weights

There were no Lotilaner-related effects on body weights or body weight changes at any dose.

Feed Consumption

There were no Lotilaner-related effects on food consumption at any dose.

Ophthalmoscopy and Tonometry

1) Gross ocular observation of the eye reactions for hyperemia, swelling and discharge scoring was performed once during the pre-dose period, on Day 1 of dosing (2 hours ± 1 hour after last dose), prior to the first dose on Day 2, weekly during the first month prior to the first dose of the day, then once every two weeks prior to the first dose of the day (relative to the end of dosing of each group).

There were no Lotilaner-related gross ocular findings (redness, swelling or presence of discharge) at any dose.

2) Ophthalmic examinations were conducted by a Board-Certified Veterinary Ophthalmologist using an indirect ophthalmoscope, a slit-lamp biomicroscope, and a tonometer for intraocular pressure (IOP) measurements. They were performed on all animals once during the pre-dose period and once towards the end of Weeks 5, 8, 13, 27 and 39.

There were no Lotilaner-related ophthalmological findings or effects on IOP at any dose.

Electroretinography (ERG)

ERGs were recorded from all animals once during the pre-dose period, and towards the end of dosing period (Week 38/39).

Each ERG occasion was performed as *per* Study Specific Procedure and consisted of the following series of stimuli:

Step	Log scale (dB)	Number to average	Time between flashes
Scotopic Phase – following dark adaptation			
1	-30	5	10 seconds
2	-10	5	15 seconds
3	0	2	Minimum 2 minutes
Photopic Phase – following adaption to background light (25 to 30 cd/m^2) for at least 5 minutes			
4	0	20	1 second (1 Hz)
5	0	20	0.034 seconds (29.4 Hz)

Steps 1 to 3 were used to evaluate scotopic luminance response. After step 3, animals were subjected to light adaptation, using the Ganzfeld background light for at least 5 minutes. Steps 4 and 5 allowed the evaluation of the latency and amplitude of the cone response. The time between flashes allowed the retina to recover its sensitivity. Baselines were corrected as necessary to correct waveform drift. Waveforms were analyzed for a-wave, b-wave and flicker amplitudes and implicit times/latency, where possible.

Group comparisons were performed with Dunnett's and Dunn's tests.

There were no apparent Lotilaner-related effects on scotopic or photopic a- or b-wave amplitudes or implicit times.

Electrocardiography (ECG)

Manual ECG traces (limb leads I, II and III, and augmented leads aVR, aVL, and aVF) were obtained from all animals once during the pre-dose period and once towards the end of the dosing period (Week 38/39). ECGs were evaluated by a board-certified veterinary cardiologist. QT was corrected using the Van de Water's formula. QTc = QT - 0.087 ((60/HR)-1) which was acceptable.

There were no effects in ECG recordings compared to pre-treatment or to the control group at any dose.

Clinical Pathology

Clinical pathology evaluations (hematology, clinical chemistry, coagulation, and urinalysis) were performed on all animals once during the pre-dose period and on all animals once towards the end of the dosing period (Week 38/39).

There were no Lotilaner-related effects on hematology, coagulation, clinical chemistry, or urinalysis parameters.

Hematology and Coagulation

Parameters evaluated
red blood cell (erythrocyte) count
hemoglobin
hematocrit
mean corpuscular volume
mean corpuscular hemoglobin
mean corpuscular hemoglobin concentration
red cell distribution width
absolute reticulocyte count
platelet count
white blood cell (leukocyte) count
absolute neutrophil count
absolute lymphocyte count
absolute monocyte count
absolute eosinophil count
absolute basophil count
absolute large unstained cell count
blood smear
prothrombin time
fibrinogen
activated partial thromboplastin time

Clinical Chemistry

Parameters evaluated:
glucose
urea nitrogen
creatinine
total protein
albumin
globulin
albumin: globulin ratio
total cholesterol

triglycerides
total bilirubin
aspartate aminotransferase
alanine aminotransferase
alkaline phosphatase
gamma glutamyltransferase
creatine kinase
calcium
inorganic phosphorus
sodium
potassium
chloride

Urinalysis
Parameters evaluated:
appearance (clarity and color)
volume
specific gravity
pH
protein
glucose
ketones
bilirubin
blood
microscopic examination of sediment

Gross Pathology
Animals surviving to scheduled termination were euthanized on Day 274 (Main) or Day 302 (Recovery) except for Animal 4003 euthanized at Day 37 that underwent histopathological examination since animal was moribund, severely weak, and in deteriorating condition.

No Lotilaner-related macroscopic findings were noted at the Terminal or Recovery sacrifice.

Organ Weights
Organ weights, as indicated below, were recorded at each scheduled sacrifice. Paired organs were weighed together.

Adrenals	Ovaries	Testes
Brain	Pituitary	Thymus
Heart	Prostate	Thyroids with parathyroids
Kidneys	Spleen	Uterus
Liver		

Bone marrow smears were prepared from the femur of each animal at scheduled sacrifices but were not evaluated.

There were no organ weight changes considered related to the administration of Lotilaner at Terminal and Recovery necropsies.

Histopathology

The following tissues (when present) from each animal were preserved in 10% neutral-buffered formalin, unless otherwise indicated.

Abnormal findings	Heart [3]	Sciatic nerve
Adrenals	Ileum	Skeletal muscle (thigh)
Animal identification (microchip) [1]	Jejunum	Skin, subcutis & mammary gland (inguinal) [7]
Aorta (thoracic)	Kidneys	Spinal cord (cervical)
Brain [2]	Lacrimal glands	Spleen
Cecum	Liver (2 lobes)	Sternum with marrow
Colon	Lungs (2 lobes) with bronchi [4]	Stomach
Duodenum	Lymph nodes (mandibular and mesenteric)	Testes [6]
Epididymides	Nictitating membranes	Thymus
Esophagus	Optic nerves (additional portion)	Thyroids with parathyroids [8]
Eyes (including a portion of the optic nerve) [6]	Ovaries	Tongue
Eyes lids (upper and lower)	Pancreas	Trachea
Extraocular muscles [9]	Pituitary	Urinary bladder
Femur with marrow	Prostate	Uterus [5]
Gallbladder	Rectum	Vagina
	Salivary gland (mandibular)	

1. Fixation and preservation only.
2. Cerebral cortex, midbrain, cerebellum and medulla. Trimmed as per *Bolon B. et al. (2013)*.
3. Section including both ventricles and atria, septum with papillary muscle.
4. Lungs were infused with formalin.
5. Horns, body and cervix.
6. Fixed in Davidson' solution.
7. Mammary gland was examined histologically only when present in routine sections.
8. Parathyroids were examined histologically only when present in routine sections.
9. Rectus dorsalis, rectus medialis, rectus ventralis, and rectus lateralis.

Tissues were examined for all Main and Recovery animals of Groups 1 (Control) and 4 (High Dose), at unscheduled euthanasia, and any gross abnormalities from animals in all groups.

Detailed ocular histopathology was performed from both eyes for all Main and Recovery animals of Groups 1 (Control) and 4 (High Dose), and at unscheduled euthanasia: eye globe, optic nerves (longitudinal and transverse sections), extraocular muscles, eyelids (upper and lower), lacrimal gland, and nictitating membrane were evaluated. Five sagittal sections of each eye were prepared, with at least one section taken through the equivalent of the macula/fovea. Paraffin blocks and slides from both eyes of Main animals of intermediate groups were prepared.

Adequate Battery: No. Nasal turbinate and nasopharynx are missing from this list. These are important organs to observe under the microscope since the test compound accumulated in tears.

Peer Review:
Not indicated in the report.

No Lotilaner-related microscopic findings were noted at the Terminal or Recovery sacrifice.

Special Evaluation
NA

Toxicokinetics
Blood samples were collected from all animals on Days 1 and 273 prior to any subsequent dosing at pre-dose, and at 1, 5, 9, 12, and 24 hours after the first dose of the day. Additionally, blood samples were collected from all animals on Days 196 (prior to first dose), 210 (prior to first dose), 224 (prior to first dose) and 302 (prior to termination).

In general, sex differences in Lotilaner mean C_{max} and AUC_{0-24} values were less than 2-fold indicating that there was no toxicokinetic differences between the sexes.

Exposure Ratios for C_{max} and AUC_{0-24} were slightly less than dose proportional on Day 1 and Day 273. The decrease in proportionality was <50% and not considered significant.

Lotilaner mean C_{max} and AUC_{0-24} values were higher on Day 273 than on Day 1 for Groups 2 through 4, indicating accumulation of Lotilaner after repeat administration in dogs. Mean accumulation ratios values slightly increased with increasing dose for C_{max} and remained similar for all AUC_{0-24}. Mean accumulation in plasma was 24 to 27-fold from Day 1 to Day 273.

Groups 2 through 4 generally had similar C_{max}/Day and AUC_{0-24}/Day. On Day 1 they ranged from 0.0126 to 0.0209 ng/mL/µg for C_{max}/Day and 0.239 to 0.393 h*ng/mL/µg

AUC_{0-24}/Day. Day 273 values ranged from 0.312 to 0.535 ng/mL/µg and 6.39 to 10.2 h*ng/mL/µg, for C_{max}/Day and AUC_{0-24}/Day, respectively.

In the females, C_{max} and AUC_{0-24} on Day 273 at the high dose of (b) (4) µg/eye/day reached 369 ng/mL and 7,290 h*ng/mL, respectively. In the males, C_{max} and AUC_{0-24} on Day 273 reached 436 ng/mL and 8,290 h*ng/mL, respectively.

The 4-week recovery period after the final dose on Day 273, generally showed a minimum of 2-fold drop in concentration. Indicating that a longer recovery period was needed to clear lotilaner.

> **This reviewer's comment:** Note that $T_{1/2}$ was not reported in this study due to "an inability to characterize the elimination phase" (*per* the Sponsor). As was the case with the rabbit study, the recovery period in the dog study was not 5 half-lives as recommended, but it was acceptable since no toxicity was found.

7 Genetic Toxicology

From the review of IND143686[12]:
AHC 2224920 was negative for genotoxic potential in a complete battery of genetic toxicology studies performed under GLP standards (i.e*., an Ames*, an *in vitro* chromosomal aberration assay in cultured peripheral human lymphocytes, and an *in vivo* micronucleus assay in rats).

8 Carcinogenicity

As agreed with the Sponsor during their pre-NDA interaction with the Division, carcinogenicity studies were not conducted to support the approval of Lotilaner Ophthalmic Solution, 0.25%, for the treatment of Demodex blepharitis. Instead, Tarsus submitted a waiver request for carcinogenicity studies for the indication sought. A summary of the Sponsor's justifications and this reviewer's evaluation is reproduced herein. The original document is copied in **Appendix 1 including the references in support of the waiver request.**

The carcinogenicity risk assessment was conducted by considering 1) the mechanism of action of Lotilaner, 2) evaluating literature and regulatory agency assessment reports for potential carcinogenic effects of Lotilaner and related isoxazolines (e.g., cellular proliferation, evidence of preneoplastic or neoplastic lesions, immunotoxicity, tumor

immune-surveillance, and induction of oncogenic viruses), 3) analyzing the nonclinical toxicology studies conducted with Lotilaner for evidence of cellular proliferation or other early hallmarks of cancer as well as 4) pharmacology studies for expected binding to various receptors, enzymes, transporters, and ion channels that may be involved in carcinogenic processes, and 5) evaluating clinical safety data for malignancy risk.

> **This reviewer's comment:** Based on ICHS1A[13], this reviewer agreed with the Sponsor's criteria for evaluating the need for carcinogenicity studies with Lotilaner in the ocular indication.

---

[13] https://www.fda.gov/media/71921/download

1) Lack of observed carcinogenicity in veterinary use:

The Sponsor indicated that "Lotilaner has been approved for use as an ectoparasiticide for veterinary medicine in the EU and US since 2017 and 2018, respectively. Reviews of a post-marketing pharmacovigilance assessment and current literature did not identify data suggesting that Lotilaner elicits carcinogenesis in companion animals." Further, "The initial European Public Assessment Report for Credelio notes that carcinogenicity studies were not required for veterinary approval due to lack of genotoxic potential, lack of structural alerts, and lack of neoplastic lesions in repeat dose toxicity studies (CVMP, 2017)."

> **This reviewer's comment:** The period since the approval of the veterinary product is too short to evaluate for carcinogenicity in animals based on post-marketing pharmacovigilance this far. Further, the requirements for approval of a veterinary drug are likely less stringent than for a human drug. Nevertheless, Sponsor's arguments favor a noncarcinogenic potential for Lotilaner.

2) Lack of pre-neoplastic or neoplastic findings in systemic toxicity studies in rats and dogs:

Sponsor conducted a "26-Week Oral Gavage Toxicity and Toxicokinetic GLP Study in Rats with a 4-Week Recovery Period" (Study 8454872) and a "Pivotal Eight-Month Target Animal GLP Safety Study of AHC 2224920 in 8-Week-Old Beagle" (Study (b) (4)-13-010).

In the 26-week study 8454872 in the Han Wistar rat, reviewed under IND (b) (4)[14,15], Lotilaner administered orally at 5, 15, and 30 mg/kg/day was well tolerated at all doses tested. There was a test-article related dose dependent increase in the incidence and severity of adrenal cortical hypertrophy, which the Sponsor attributed to physiological stress. The NOAEL was 30 mg/kg/day. This dose level corresponded to mean C_{max} and AUC values of 73,300 ng/mL and 1,645,000 h*ng/mL, respectively, in males and females combined.

In the 8-month study ((b) (4)-13-010) in the Beagle dog reviewed under IND143686[12], the oral administration of AHC 2224920 up to 215 mg/kg, one day per month, for eight months to male and female 8-week-old dogs was found to be well tolerated. The NOAEL was 215 mg/kg. C_{max} and AUC_{0-672h} achieved at the NOAEL in male and female combined were 21,300 ng/mL, and 9,870,000 ng.h/mL.

Further, the exposures observed at the NOAEL in the 6-month oral rat study and the NOEL of the 8-month oral dog study provided large exposure multiples over the clinical systemic exposures after topical ocular administration of TP-03, on an AUC basis. There were no signals or apparent risk of carcinogenicity identified in the described chronic rat or dog studies.

> **This reviewer's comment**: Although Sponsor's arguments were well received and support a weight of evidence approach, Studies 8454872 and (b) (4)-13-010 were not a substitute for actual carcinogenicity studies based on ICH S1B[16].

---

[14]https://darrts.fda.gov/darrts/faces/ViewDocument?documentId=090140af8067e1c0
[15]https://darrts.fda.gov/darrts/faces/ViewDocument?documentId=090140af8062d217
[16] https://www.fda.gov/media/71935/download

3) Lack of significant *in vitro* binding on human receptors of interest

This included lack of binding the pharmacological target as Lotilaner is primarily directed against insect and acarine GABA mediated chloride channels.

Lotilaner (10 µM) was tested for off target binding against a wide range (133) of receptors, transporters, enzymes, and ion channels with a threshold for significant off-target activity of 50% *in vitro*. None of the targets exhibiting >50% binding activity were directly associated with carcinogenesis.

> **This reviewer's comment:** This reviewer agreed with Sponsor's argument.

4) Lack of genotoxicity findings

Lotilaner was negative for genotoxic potential in a complete battery of GLP genetic toxicology studies

> **This reviewer's comment:** This reviewer agreed with Sponsor's conclusion.

5) Lack of a class effects on carcinogenesis with related compounds

Isoxazoline compounds that are currently approved for veterinary use as antiparasiticides including afoxolaner [NexGard, FDA-approved since May 2013], fluralaner [Bravecto, FDA-approved since 2014] and sarolaner [Simparica, FDA-approved since 2016]) have the same pharmacology as Lotilaner, i.e., are highly insect-selective blockers of GABA-gated Cl channels with lower potency against insect glutamate-gated Cl channels. None of these compounds have been tested for carcinogenicity potential which is not a requirement for veterinary drugs as they are not genotoxic and did not induce preneoplastic lesions in repeat dose toxicity studies. There have been no reports thus far of an increased risk of cancer during long-standing veterinary use of these products.

> **This reviewer's comment:** The period since the approval of the veterinary product is too short to evaluate for carcinogenicity in animals based on post-marketing pharmacovigilance this far. Further, the requirements for approval of a veterinary drug are likely less stringent than for a human drug.

6) Lack of findings in clinical trials this far consistent with a test article related effects on cell proliferation

To date, there is no evidence of an increased risk of malignancies in subjects treated for up to 43 days in clinical trials evaluating TP-03, 0.25%. In addition, TP-03 is not intended to be used chronically in the clinic, which further reduces carcinogenicity risk concerns.

> **This reviewer's comment:** This reviewer agreed with Sponsor's conclusion as the product indication is for 6 weeks treatment, and hence is not chronic.

Based on the totality of Sponsor's arguments, this reviewer judged the waiver request for carcinogenicity studies receivable since the weight of evidence indicated no cause for concerns in the animals or humans, nor significant systemic exposure for a long period of time that would justify evaluating the cancer risk with Lotilaner Ophthalmic Solution, 0.25%, for the indication sought.

9 Reproductive and Developmental Toxicology

From the review of IND143686[12]:

- In an embryo-fetal toxicity in the rat ((b) (4)-12-036) by oral gavage with AHC 2224920 at the doses of 9, 18 and 50 mg/kg/day, the NOAEL for maternal and fetal toxicities was 18 mg/kg/day, equivalent to a safety margin of (b) (4) X with the MRHD of (b) (4) mg/kg for a 60 kg human. No TK data were provided.

Key results
Oral gavage administration of AHC 2224920 at a dose level of 50 mg/kg/day to pregnant Wistar (Han) rats during the period of organogenesis was associated with marked body weight loss and associated clinical signs that resulted in 11 females being terminated prematurely. For surviving females there was statistically significantly reduced overall body weight gain and lower food consumption throughout the dosing period.

Following administration of AHC 2224920 at 18 and 50 mg/kg/day, there were 1 and 3 females, respectively with 100% implantation loss at necropsy on Gestation Day 20. This finding was treatment-related at 50 mg/kg/day, but at 18 mg/kg/day, the finding fell within the historical control range at this laboratory and in the absence of other adverse effects, was not considered adverse.

The NOAEL for maternal toxicity following administration of AHC 2224920 was 18 mg/kg/day. There were only 7 litters for evaluation at 50 mg/kg/day and this was a maternally toxic dose level. There was a marked effect on mean fetal weight at 50 mg/kg/day and there was an indication of a developmental delay characterized by an increased number of fetuses with incomplete ossification of individual bones of the skeleton. One fetus at 50 mg/kg/day AHC 2224920 had situs inversus of the thoracic and abdominal viscera and 1 fetus at 18 mg/kg/day had an absent eye. These are serious and rare malformations which could not be confirmed to be unrelated to treatment in the absence of historical control data (not provided at the IND submission). Such historical control data should be required at the marketing application.

> **This reviewer's comment:** Historical Control Laboratory Standard Data for the Crl:WI(Han) Rat from 2008 to 2013 for reference purposes including 231 Litters and 2283 Fetuses were included with the NDA submission. There was 0% incidence of fetus with situs inversus of the thoracic and abdominal viscera in the historical control data versus 1.3% fetus in study (b) (4)-12-036 (1 fetus in 1 litter) and 0.9% maximum incidence of absent eye in the historical control data versus 0.7% in study (b) (4)-12-036 (1 fetus in 1 litter). This reviewer considered the malformation situs inversus test article related.

- In a 2-generation study in the rat ((b) (4)-13-008) by oral gavage with AHC 2224920 at the doses of 1, 5 and 40→20 mg/kg/day, the NOAEL for maternal and fetal toxicities was 5 mg/kg/day equivalent to a safety margin of (b) (4)X with the MRHD of (b) (4) mg/kg for a 60 kg human. No TK data were provided.

Key results
Daily administration of AHC 2224920 was well tolerated in both males and females at dose levels of up to 40 mg/kg/day for 10 weeks before pairing. After pairing, females at the highest dose showed reduced pregnancy rate and low implantation rates, both associated with low body weight gain and low food consumption. Halving the dose level administered to the high dose animals to 20 mg/kg/day appeared to improve the pregnancy rate of the animals, which ultimately mated later, however litter sizes were still low.

No F1 generation was evaluated at the high (40→20 mg/kg/day) dose level due to test-article related low fertility of females in the F0 generation.

At the mid and low doses, there were no adverse effects on the F1 generation. At 5 mg/kg/day, there was slightly increased testis weight for males and increased sperm velocity that did not impact fertility. Male body weights were also slightly increased compared to control in the F1 generation. Slightly lower mean body weight during lactation for the F2 pups at 5 mg/kg/day was associated with a slight delay in the attainment of righting reflex and pinna detachment, but later development during weaning was unaffected.

Mating and fertility of the F1 were unaffected, indicating the minor pathological changes observed in utero were not of biological significance A dose level of 5 mg/kg/day was the NOAEL in this study.

- There was no designated fertility study conducted, however the results of the 2-generation study suggested that the NOAEL for male fertility was 20 mg/kg/day, which provides a safety margin of (b) (4)X with the MRHD of (b) (4) mg/kg for a 60 kg human.

9.2 Embryonic Fetal Development

Study title: Oral Gavage Embryo-Fetal Development Study Including Toxicokinetics with Lotilaner in Rabbits

Study no.:	8447423
Study report location:	eCTD0001
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 17, 2020
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lotilaner, lot # 1950J013, 99.9% purity

Key Study Findings

Pregnant New Zealand White (NZW) rabbits (n=20/group) were administered 0, 2.5, 7.5, or 20 mg/kg/day Lotilaner by oral gavage during the period of organogenesis (GD 7 through 19). Decreased food consumption during the dosing phase was noted for animals administered 20 mg/kg/day. While the magnitude of reduced mean food consumption was small, one animal administered 20 mg/kg/day demonstrated severely reduced food consumption accompanied by body weight loss requiring unscheduled moribund sacrifice. It is the opinion of this reviewer that the demise of this animal was test-compound related.

In all other animals, no additional Lotilaner-related effects were observed on clinical observations, mean body weight, macroscopic observations, reproductive performance, cesarean section parameters, or fetal evaluations at any dose level.

The maternal NOAEL was 7.5 mg/kg/day (C_{max} = 3,200 ng/mL; AUC_{0-24} = 56,200 h*ng/mL) and the fetal NOAEL was 20 mg/kg/day (C_{max} = 8,680 ng/mL; AUC_{0-24} = 170,000 h*ng/mL). These NOAELs provided safety margins of (b) (4) for maternal and fetal toxicities, respectively, with the MRHD of (b) (4) mg/kg for a 60 kg human.

Methods

Doses:	2.5, 7.5, 20.0 mg/kg
Frequency of dosing:	Daily
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	20% Cremophor EL (v/v)/ 80% Deionized Water (v/v)

Species/Strain:	New Zealand White rabbit
Number/Group:	20 females
Satellite groups:	No
Study design:	See study design below
Deviation from study protocol:	None of impact

Study design

Group[a]	Number of Mated Females	Dose Level[b] (mg/kg)	Dose Concentration[b] (mg/mL)	Animal Numbers
Toxicity Animals[c]				
1 (Control)	20	0.0	0.0	B0001-B0020
2 (Low)	20	2.5	0.5	B0101-B0120
3 (Mid)	20	7.5	1.5	B0201-B0220
4 (High)	20	20.0	4.0	B0301-B0320

a Group 1 was administered vehicle control article only.
b Animals were dosed at a volume of 5 mL/kg.
c Three toxicity animals/group/time point were also used for collection of toxicokinetic (TK) blood samples.

(Copied from Study 8447423 on p 14)

Animals were dosed once daily beginning on gestation day (GD) 7 and continuing through GD 19.

Observations and Results

Mortality

One Animal (B0320) administered 20 mg/kg/day was sacrificed at an unscheduled interval on GD 19 following 6 days of low food consumption, body weight loss >10%, minimal fecal output, lethargy, and low body weight. Although one instance of unscheduled study removal was within the range of Historical Control Data (HCD) for the NZW rabbit, as this event occurred at the high dose, was not accidental, and less severe reduction in food consumption was noted for other animals administered 20 mg/kg/day, an effect of Lotilaner could not be dismissed. The Sponsor concluded the unscheduled death of Animal B0320 was of uncertain relationship to the test article.

> **This reviewer comment:** In a preliminary dose range finding study at 15, 50, and 150 mg/kg by oral gavage in the rabbit where animals were dosed from GD 7 to GD 19 (Study 8447422 not reported in this document), the dose of 15 mg was the Maximum Tolerated Dose (MTD) with 1/6 animal in this group showing continuously low food consumption and removed from the study on GD 18. Above the MTD, animals perished with signs of low food consumption and loss of body weight. Similar Lotilaner toxicity was observed in the rat too. It is this reviewer's opinion that the death of Animal B0320 was test article related.

Clinical Signs

No test-article related clinical signs were observed during the study except for animal B0320.

Body Weight

Animal B0320 (administered 20 mg/kg/day) was noted with severe body weight loss from GD 14 to 18 (12.8% of total body weight lost). This corresponded with a period of 5 days of severe reduction in food consumption for this animal (1 g/day consumed on average). The severe effect on body weight for this animal required unscheduled sacrifice.

In all other test-article treated animals there were no statistically significant differences with control for body weight and body weight gain during the study.

Feed Consumption

During the dosing phase (GD 7 to 20), mean food consumption for controls was 1654 grams. Mean food consumption for animals administered 2.5, 7.5, or 20 mg/kg/day was altered by +3.3, -4.0, or -12.8%, respectively, from control levels. During the post dosing period (GD 20-29) food consumption for animals administered 20 mg/kg/day was increased 9% relative to controls, including a statistically significant increase on GD 27.

Reduced food consumption during the dosing phase for animals administered 20 mg/kg/day was considered test article-related due to statistically significant differences in food consumption on GD 7 and GD 14, persistent notable decreased food consumption from GD 13 through 16, and compensatory increased food consumption in the post-dosing phase. Increased food consumption in the post-dosing phase indicated that decreased mean food consumption for animals administered 20 mg/kg/day was a transient effect of dosing. Due to the small magnitude of reduced food consumption and the lack of effect on body weight or body weight gain, reduced mean food consumption was considered non-adverse.

Toxicokinetics

Exposure, as assessed by lotilaner C_{max} and AUC_{0-24}, increased with the increase in dose level from 2.5 to 20 mg/kg. The increases in C_{max} and AUC_{0-24} values were dose proportional (Table 14).

Table 14: Summary of Lotilaner Toxicokinetic Parameters in Pregnant Rabbits on GD 13

Dose Group	Dose Level (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-24} (h*ng/mL)
2	2.5	915	8.00	18900
3	7.5	3200	4.00	56200
4	20	8680	4.00	170000

Note: Due to the lack of a distinct elimination phase, half-life ($t_{1/2}$) was not reported.

(Copied from Study 8447423 on p 23)

Dosing Solution Analysis

All samples met acceptance criteria for homogeneity and concentration verification.

Terminal Evaluations

Macroscopic Evaluation

There was no test article related effects on macroscopic observation.

Reproductive Performance

One animal (Animal B0120) administered 2.5 mg/kg/day and one animal (Animal B0307) administered 20 mg/kg/day were found to be not pregnant. Pregnancy was confirmed in all other animals at necropsy.

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

No effect of lotilaner was noted on cesarean section parameters.

Fetal Evaluations (Malformations, Variations, etc.)

External Evaluations

No effect of lotilaner was noted on fetal external malformations. No external variations were observed.

Fresh Visceral Evaluations

No effect of lotilaner was noted on fetal visceral malformations or variations.

The visceral variation oviductal cyst was observed in one control fetus, two fetuses from an animal administered 7.5 mg/kg/day, and one fetus from an animal administered 20 mg/kg/day. The incidence of oviductal cyst was elevated for animals administered 7.5 mg/kg/day (Litter incidence = 10%; Fetal incidence = 1.55%) relative to controls (Litter incidence = 5%; Fetal incidence = 1%) and historical control data (HCD) (HCD max Litter incidence = 5%; Fetal incidence = 1.59%). There was no evidence of a dose response for this finding; the incidence was not increased in litters administered 20 mg/kg/day (Litter incidence = 6%; Fetal incidence = 0.93%) *versus* at 7.5 mg/kg/day. Rather, incidence in

controls and litters administered 20 mg/kg/day was similar. The Sponsor concluded it was incidental. This reviewer agrees with the Sponsor.

Skeletal Evaluations
No effect of lotilaner was noted on fetal skeletal malformations or variations.

The skeletal variation supernumerary lumbar vertebra was observed in one control fetus, one fetus receiving 2.5 mg/kg/day, five fetuses receiving 7.5 mg/kg/day, and one fetus from an animal administered 20 mg/kg/day. The incidence of the skeletal variation supernumerary lumbar vertebra was elevated in litters from animals administered 7.5 mg/kg/day (Litter incidence = 10%; Fetal incidence = 2.67%). The incidence of this variation in the group administered 7.5 mg/kg/day was outside the range of HCD (HCD max: Litter = 5%; Fetal = 0.65%. Note: due to a classification change, this variation is listed as a malformation in the attached HCD). The incidence of this variation in animals administered 20 mg/kg/day was like controls (Litter incidence = 5%; Fetal incidence = 0.63% in control *versus* Litter incidence = 6%; Fetal incidence = 0.51% at 20 mg/kg), indicating that the effect was not dose responsive. Due to the lack of dose-response, the elevated incidence of supernumerary lumbar vertebra in animals administered 7.5 mg/kg/day was considered incidental. This reviewer agreed with the Sponsor.

10 Special Toxicology Studies

From the review of IND143686[12]:

- AHC 2224920 was a minimal ocular irritant after instillation of 100 mg into the left conjunctival sac of male New Zealand White rabbits for 3 days.
- AHC 2224920 triggered no alert for respiratory (including occupational asthma) and skin sensitization after analysis using Derek Nexus.
- AHC 2224920 was classified as non-irritant to rabbit skin according to the Draize classification system.
- The acute median lethal dermal dose of AHC 2224920 to rats was found to exceed 2000 mg/kg by acute dermal toxicity test.
- In a Local Lymph Node Assay in the mouse, ACH 2224920 did not have the potential to cause skin sensitization.

11 Integrated Summary and Safety Evaluation

Lotilaner is an ectoparasiticide and insecticide that was initially developed as a veterinary product for control of flea and tick infestations and has also been found effective against Demodex mites. The nonclinical safety of Lotilaner and Lotilaner Ophthalmic Solution,

0.25% were developed in the rabbit, rat and dog. Isoxazolines, including lotilaner, are specific inhibitors of (GABA)-gated chloride channels (GABACIs) paralyzing the nervous system of insects or arachnids leading to their death. Lotilaner is not an inhibitor of mammalian GABACIs.

Microscopic findings related to treatment with AHC 2224920 were noted in body weight, skin/mucosa, ovary, lung, adrenal, pituitary (males only) and thymus when administered orally to rats for 13 weeks consecutively. Mortality due to early sacrifice of moribund animals occurred at 60 mg/kg/day in females and at ≥40 mg/kg/day in males. Safety margins for these findings were very large and not clinically significant for the intended indication (Table 15).

Table 15: Nonclinical Findings with Oral Studies in Rats

Toxicity/Finding	Reversible?	Species/ Duration	Dose (mg/kg/day)	Clinical Safety Margin (Based on MRHD = (b) (4) mg/kg/day)
Mortality/Moribundity		Rat/13 weeks	40	(b) (4)
Body weight loss	Yes	Rat/13 weeks	40	
Skin/mucosal lesions	Partial	Rat/13 weeks	5	
Ovary interstitial cell vacuolation	Partial	Rat/13 weeks	20	
Lung with foamy macrophages	No	Rat/13 weeks	20	
Pituitary vacuolation	Partial	Rat/13 weeks	20	
Adrenal gland cortical hypertrophy	Yes	Rat/13 weeks	20	
Thymus involution/atrophy	Partial	Rat/13 weeks	40	

(Copied from the Pharmacology and Toxicology review of IND143686[12] on page 4)

In pivotal ocular toxicity studies in the rabbit and the dog for up to 6 and 9 months, respectively, no adverse effects were found up to the highest doses tested of (b) (4) µg/eye/day/OU or (b) (4) µg/animal/day in both species. Ocular (Table 16) and systemic (Table 17) safety margins achieved with the proposed therapeutic dose were 2.66X, and (b) (4)X (6-month rabbit study) and (b) (4)X (9-month dog study), respectively, which were acceptable.

It must be noted that none of the ocular toxicity evaluated the nasal cavities and nasopharynx, which are important organs of toxicity after ocular exposure.

Table 16: Ocular Safety Margin Based on the Ocular NOAEL in the 6- and 9-month Pivotal Toxicity Studies in the Rat and the Dog, respectively

Species	NOAEL	Total Dose (mg/eye/day)	Margins of Exposure Based on Total Dose
			(b) (4) mg/eye/day (Highest Intended Clinical Dose)
Rabbit	0.25% 2 drops ((b) (4) µL drop) *per* dose/OU TID	(b) (4)	2.66
Dog	0.25% 2 drops ((b) (4) µL drop) *per* dose/OU TID	(b) (4)	2.66

(Table made by this reviewer)

Table 17: Systemic Safety Margins Based on the Systemic NOAELs in the 6- and 9-month Pivotal Toxicity Studies in the Rat and the Dog, respectively

Nonclinical				Clinical Safety Margins (Based on Dose)
Species/ Duration	NOAEL (µg/kg/day)	HED (µg/kg/day)	C_{max} ng/mL /AUC ng.h/mL	MRHD ((b) (4) µg/kg/day)
Rabbit/ 6-month topical ocular	(b) (4)	(b) (4)	251/5385	(b) (4)X
Dog/ 9-month topical ocular	(b) (4)	(b) (4)	403/7790	(b) (4)X

NOAEL = no-observed-adverse-effect level
AUC = AUC_{0-24} = area under the concentration-time curve
MRHD = maximum recommended human dose
HED = human equivalent dose
C_{max} = maximal concentration
M = Males; F = Females
(Table made by this reviewer)

References

Snyder DE, Wiseman S and Liebenberg JE. Efficacy of lotilaner (Credelio™), a novel oral isoxazoline against naturally occurring mange mite infestations in dogs caused by Demodex spp. Parasites & Vectors (2017) 10:532.

Weber T and Selzer P. Isoxazolines: Novel Chemotype Highly Effective on Ectoparasites. ChemMedChem 2016, 11, 270 – 276.

12 Appendix/Attachments

Appendix 1
Request for a Waiver/16 pages (copied from Module 1.1.2)

16 Page(s) have been Withheld in Full as b4 (CCI/TS) immediately following this page

---

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

MURIEL J SAULNIER
05/09/2023 02:18:54 PM

KIMBERLY P HATFIELD
05/09/2023 04:21:43 PM
I concur with the review and conclusions of Dr. Saulnier. Acting on behalf of Dr. Lori Kotch