CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

217603Orig1s000

CLINICAL PHARMACOLOGY REVIEW(S)

Office of Clinical Pharmacology Review

NDA Number	217603
Link to EDR	View submission in docuBridge
Submission Date	8/25/2022
Submission Type	505(b)(1) NME NDA; Standard
Brand Name	XDEMVY™
Generic Name	Lotilaner
Dosage Form and Regimen	Ophthalmic solution, 0.25% One drop in each eye twice daily (approximately 12 hours apart) for 6 weeks
Route of Administration	Topical ocular
Proposed Indication	The treatment of *Demodex* blepharitis
Applicant	Tarsus Pharmaceuticals, Inc.
Associated IND	IND 143686
OCP Review Team	Hyewon Kim, Ph.D. Ping Ji, Ph.D.
OND Division	Division of Ophthalmology
OCP Final Signatory	Suresh Doddapaneni, Ph.D., Director (Acting), Division of Inflammation and Immune Pharmacology, Office of Clinical Pharmacology

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1 EXECUTIVE SUMMARY

The Applicant, Tarsus Pharmaceuticals, Inc., has submitted a 505(b)(1) NDA on August 9, 2022, to support the approval of a lotilaner ophthalmic solution 0.25% (proposed trade name: XDEMVY™) for the treatment of Demodex blepharitis in adults. Lotilaner is a selective inhibitor of insect and acarine γ-aminobutyric acid (GABA) mediated chloride channels. Proposed dosing regimen is one drop in each eye twice daily (BID), approximately 12 hours apart, for 6 weeks.

Lotilaner has been previously developed as an oral veterinary drug for the control of flea and tick infestations in dogs and cats (CREDELIO™) and has been shown to be effective against Demodex mites in dogs. Currently, there are no approved pharmaceutical treatments for Demodex blepharitis in humans.

The clinical pharmacology submission includes one pharmacokinetic (PK) trial (Study TRS-012), a phase 1, single-are, open-label, single-center study in healthy subjects assessing the PK and safety of lotilaner instilled in both eyes for 42 days. There are two phase 3 pivotal trials with PK data (Studies TRS-009 and TRS-010) in which PK samples were collected in a subset of participants. In addition, the submission contained seven *in vitro* studies characterizing metabolism, protein binding, partitioning in blood cells, metabolic/transporter-based drug interaction, and the effect of lotilaner on hERG current.

1.1 Recommendations

The Office of Clinical Pharmacology/Division of Inflammation and Immune Pharmacology (OCP/DIIP) has reviewed the clinical pharmacology data submitted in support of NDA 217603 for the proposed lotilaner 0.25% ophthalmic solution and found the application acceptable to support approval from a clinical pharmacology perspective.

Review Issue	**Recommendations and Comments**
Pivotal or supportive evidence of effectiveness	Primary evidence of effectiveness is based on two randomized, controlled, double-masked, parallel, phase 3 trials (Studies TRS-009 and TRS-010) in patients with Demodex blepharitis.
General dosing instructions	One drop of lotilaner ophthalmic solution, 0.25% in each eye twice daily, approximately 12 hours apart, for 6 weeks
Dosing in patients (intrinsic and extrinsic factors)	No dose adjustment is recommended for patients based on intrinsic and extrinsic factors.
Labeling	The labeling review is ongoing at the time of this review.
Bridge between the to-be-marketed and clinical trial formulations	Not applicable. There is no difference between the clinical trial formulation and the to be marketed formulation.

1.2 Post Marketing Requirement

None

2 SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

The clinical pharmacology assessment included a review of the PK data from Study TRS-012, multiple-dose PK study in healthy subjects as well as the PK findings from phase 3 pivotal trials, Studies TRS-009 and TRS-010.

2.1 Pharmacology and Clinical Pharmacokinetics

Mechanism of Action

Lotilaner is a GABA-gated chloride channel inhibitor selective for mites. Lotilaner is not an inhibitor of human GABA chloride channels.

Absorption

Following a single topical ocular administration of TP-03, 0.25% the median lotilaner whole blood lag time (T_{lag}) was 0.5 hour, indicating a delayed absorption after topical administration. Maximum systemic exposure after topical application occurs 2 hours after a single administration and by 1 hour on the last day of 42 days of treatment.
In healthy subjects, the peak concentration (C_{max}) was 0.596 ng/mL and total exposures of lotilaner 0.25%, AUC_{0-12} and AUC_{0-24}, in whole blood were 5.75 ng·hr/mL and 9.98 ng·hr/mL, respectively. These values increased after repeated ocular BID administration over 40 days, with C_{max} of 17.8 ng/mL and AUC_{0-24} of 293 ng·hr/mL.

Distribution

No distribution data for lotilaner is available in human. Lotilaner plasma protein binding was high (> 99.9%) in human plasma. The actual mean value of unbound was 0.0732 % for lotilaner (5 µM) after dialysis in human plasma. The partitioning of lotilaner to human blood cells ranged 0-20% and generally approximately closer to 10%.

Metabolism

Negligible metabolism of lotilaner was observed in blood in rats and dogs. *In vitro* study for the evaluation of lotilaner metabolic stability in human liver microsome indicated limited or no metabolism of lotilaner following 60-minute incubation. There is no lotilaner metabolism study in humans.

Excretion

The terminal plasma elimination half-life of lotilaner following BID dosing in both eyes for 40 days was approximately 1400 hours (58.3 days). However, the effective half-life, which is based on the accumulation ratio over the dosing interval of 12 hours, was 264 hours (11 days). The PK results from Study TRS-012 indicated that lotilaner systemic exposure (C_{trough}) in healthy subjects did not reach a steady state after twice daily dosing for 42 days. Per ADME studies in rats and dogs, lotilaner is primarily excreted through the biliary route whereas the renal route was a minor route of elimination.

Specific Populations

From a perspective of safety, given minimal systemic exposure following topical administration to eye, dose adjustment is not warranted in subpopulations based on the commonly known intrinsic factors.

Drug-Drug Interaction

In vitro studies showed that lotilaner is not metabolized by CYP enzymes.
In *in vitro* setting, lotilaner showed direct inhibition of a few CYP enzymes at higher concentrations than clinically relevant range. Therefore, the drug-drug interaction potential of lotilaner through CYP enzymes is minimal.

2.2 Dosing and Therapeutic Individualization

2.2.1 General Dosing

The recommended dosing regimen is one drop of lotilaner ophthalmic solution, 0.25% in each eye twice daily in the morning and in the evening, approximately 12 hours apart, for 6 weeks. This dosing regimen was evaluated in two randomized controlled clinical trials (Studies TRS-009 and TRS-010) with patients with Demodex blepharitis. These studies were designed to assess safety and efficacy, and specifically to demonstrate the effect of lotilaner 0.25% twice daily on the treatment of Demodex blepharitis.

2.2.2 Therapeutic Individualization

Therapeutic individualization is not applicable for lotilaner ophthalmic solution because it is locally administered with minimum systemic exposure.

2.2.3 Outstanding Issues

None.

2.2.4 Summary of Labeling Recommendations

The labeling review is ongoing at the time of this review.

3 COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

The proposed product is a lotilaner ophthalmic solution 0.25% (proposed trade name XDEMVY™) for the treatment of Demodex blepharitis in adults. Lotilaner is a selective inhibitor of insect and acarine GABA mediated chloride channels. Proposed dosing regimen is one drop in each eye twice daily, approximately 12 hours apart, for 6 weeks.

The proposed product was evaluated under IND 143686. A summary of key clinical pharmacology-related discussions and correspondence with the Applicant are listed in Table 3.1-1 below.

Table 3.1-1 Summary of key clinical pharmacology-related communications with the Applicant

IND 143686, Pre-IND (June 2019)	▪ The Agency agreed that the 0.25% concentration of the drug substance of TP-03 Ophthalmic Solution (lotilaner) is the maximum concentration that can be achieved with an aqueous solution and therefore, clinical testing of lower concentrations is not necessary.
Pre-NDA (June 2022)	▪ The Agency agreed that *in vitro* ADME studies, and assessment of clinical pharmacokinetics in the healthy volunteer study, TRS-012, as well as the assessment of systemic exposure after completion of drug administration in the pivotal studies (TRS-009 (Saturn-1) and TRS-010 (Saturn-2)) are adequate to characterize the pharmacokinetics of lotilaner after ophthalmic application.

Source: Reviewer's summary based on meeting minutes located in DARRTS for IND 143686

Table 3.1-2 Summary of Studies

Study Number	Study Objective	Study Design	Study Drug	Number of Subjects
TRS-012 (Hyperion)	Assess pharmacokinetics and safety of lotilaner by administration of single and multiple doses of TP-03	Single center, open-label, single arm study	One topical ocular drop of TP-03 instilled in both eyes, once or twice daily for 42 days	24
TRS-009[a] (Saturn-1)	Assessment of systemic exposure at 1 time point on Day 42	Multicenter, double masked, randomized, placebo-controlled	One topical ocular drop of TP-03 or vehicle instilled in each eye twice daily for 43 days	TP-03: 22 Vehicle: 19
TRS-010[a] (Saturn-2)	Assessment of systemic exposure at 1 time point on Day 42	Multicenter, double masked, randomized, placebo-controlled	One topical ocular drop of TP-03 or vehicle instilled in each eye twice daily for 43 days	TP-03: 114 Vehicle: 115

[a]Details of Studies TRS-009, TRS-010, TRS-012 are available at Appendix 4.

3.2 General Pharmacology and Pharmacokinetic Characteristics

Pharmacology	
Mechanism of Action	Lotilaner is a GABA-gated chloride channel inhibitor selective for mites. Inhibition of these GABA chloride channels causes a paralytic action in the target organism leading to its death. Lotilaner is not an inhibitor of human GABA chloride channels.
Active Moieties	Lotilaner
QT Prolongation	Lotilaner 600 mg as a single oral dose had no significant QTcF prolongation effect in the QTc assessment of data collected in the dedicated tQT study. The concentration is well above the plasma concentrations detected in the clinical PK study. Thus, the risk of QT prolongation with topical ocular administration of lotilaner appears low.
General Information	
Bioanalysis	The method for analysis of lotilaner in human whole blood has been adequately validated in report 8449-527.
Healthy Subjects vs Patients	The systemic exposure at the end of treatment in subjects with Demodex blepharitis was comparable with that in healthy subjects.
Dose Proportionality	Not determined
Accumulation	Estimated accumulation ratio for the exposure (AUC) of lotilaner after multiple dosing ranged 32-37 based on the long elimination half-life ($t_{1/2}$=1400 hours) and proposed twice daily dosing.
PK Variability (%)	C_{max}: 51.7% (Day 1) and 53.4% (Day 42) $AUC_{0-\tau}$: 48.5% (Day 1) and 52.2% (Day 42)
Absorption	
Median T_{max} (hours)	2 hours (Day 1) and 1 hours (Day 42)
Distribution	
Protein Binding	> 99.9%
Substrate Transporter System (*in vitro*)	Lotilaner was not a substrate of OCT1, OATP1B1, OATP1B3, P-gp, BCRP, and BSEP.
Elimination	
Terminal Half-life	1400 hours (58.3 days)
Effective Elimination Half-life	264 hours (11 days) when it is given twice daily
Metabolic Pathway	*In vitro* studies showed that lotilaner is not metabolized by CYP enzymes. Non-clinical studies in rats, cats and dogs demonstrated that lotilaner is excreted in bile as mostly unmetabolized drug.
Inhibitor/Inducer	In *in vitro* setting, lotilaner showed direct inhibition of a few CYP enzymes with IC_{50} values that are > 500 times higher than the unbound concentrations at clinical setting.
Excretion Pathway	Not determined in human. Lotilaner is primarily excreted through the biliary route in rats and dogs.

3.3 Clinical Pharmacology Review Questions

3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

As the site of drug delivery and action for lotilaner ophthalmic solution is the eye, the extent of systemic exposure does not correlate with its efficacy. Therefore, the clinical pharmacology program does not provide supportive or pivotal evidence of effectiveness.

3.3.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes. The proposed strength and dosing regimen are a lotilaner 0.25% ophthalmic solution and one drop will be instilled into each eye twice daily, approximately 12 hours apart, for 6 weeks. In the pre-IND meeting held June 2019, the Agency agreed that the 0.25% lotilaner concentration of TP-03 ophthalmic solution is the maximum concentration that can be achieved with an aqueous solution and therefore, clinical testing of lower concentrations is not necessary. Proposed dose and dosing regimen was efficacious for the treatment of Demodex blepharitis as demonstrated in two phase 3 pivotal trials. Refer to the clinical and statistical reviews for more information on efficacy and safety assessment.

3.3.3 Is an alternative dosing regimen and/or management strategy required for subpopulations based on intrinsic factors?

An alternative dosing regimen is not needed. As the site of drug delivery and action for lotilaner ophthalmic solution is the eye, the extent of systemic exposure does not correlate with its efficacy. From a perspective of safety, given minimal systemic exposure following topical administration, dose adjustment is not warranted in subpopulations based on the commonly known intrinsic factors.

3.3.4 Are there clinically relevant food-drug or drug-drug interactions, and what is the appropriate management strategy?

The drug product is an ophthalmic solution; therefore, the issue of a food-drug interaction is not relevant. *In vitro* studies showed that lotilaner is not metabolized by CYP enzymes. Non-clinical studies in rats, cats and dogs demonstrated that lotilaner is excreted in bile as mostly unmetabolized drug.
In *in vitro* setting, lotilaner showed direct inhibition of CYP2C8, CYP2C9, CYP2C19, and CYP2D6 with IC_{50} values (uncorrected for microsomal protein binding) of 0.402, 3.72, 1.56, and 4.70 µM, respectively. Of note, the observed maximum concentration (C_{max}) in all clinical trials is 46.1 ng/mL. Based on the clinical results, the estimated unbound C_{max} is 0.461 ng/mL (0.00078 µM) with conservative assumption of 1% free drug (protein binding is 99.9% bound). Therefore, the drug-drug interaction potential of lotilaner through CYP enzymes is minimal.

4 APPENDICES: INIDIVIDUAL STUDY REPORT

4.1 Phase 1 Trial: Study TRS-012

Title: "Pharmacokinetic Study to Evaluate the Whole Blood Pharmacokinetics of TP-03 Following Six Week Topical Ocular Administration"

The Applicant conducted a single center, open-label, single-arm study (Study TRS-012) in healthy subjects to evaluate the systemic lotilaner pharmacokinetic characteristics. The study enrolled 24 healthy subjects and all participants received lotilaner ophthalmic solution, 0.25% (TP-03, 0.25%). A single drop of TP-03 0.25% ophthalmic solution was instilled in each eye on the morning of Day 1 and then twice daily (in the morning and in the evening, approximately 12 hours apart) starting on Day 2 for 40 consecutive days (Days 2 to 41). The doses of Days 1, 2 (morning), 41 (evening), and 42 were self-administered under supervision of the site staff at the clinical site. All remaining doses were self-administered at home.

Study TRS-012 enrolled healthy males and females who are aged at least 18 years and a body mass index (BMI) of 18.5 - 30.0 kg/m^2, inclusive. Subjects with presence or history of significant gastrointestinal, liver, kidney disease, or surgery that could affect drug bioavailability, with history of significant cardiovascular, pulmonary, hematologic, neurological, psychiatric, endocrine, immunologic, or dermatologic disease were excluded from the enrollment. No prescription drugs except hormonal contraceptives or hormone replacement therapy were allowed from 28 days prior to the first study drug administration to the completion of the study.

4.1.1 Sample Collection and Pharmacokinetic Assessments

Blood samples for PK of each treatment were collected prior to dosing and at 0.5, 1, 2, 3, 4, 8, 12, 24 (that is, Day 2 or Day 43) hours after dosing on Day 1 and Day 42. Additional PK samples were collected prior to the morning dose on Days 2, 7, 14, 21, 28, 35, 39, 45, 49, 56, 70, 84, 98, 112, 126, 140, and 154 after the first dose. Subjects were confined to the clinical site from at least 10 hours before the morning drug administration of Day 1 and Day 42 until at least 24 hours after administration of study drug (Days -1 through 2 and Days 41 through 43). Remaining samples were collected on an outpatient basis. All samples were collected using tubes containing dipotassium ethylenediaminetetraacetic acid (K_2EDTA).

PK samples were analyzed using a validated high-performance liquid chromatography-mass spectrometry-mass spectrometry (HPLC/MS/MS) method to determine the concentration of lotilaner in human plasma with a lower limit of quantitation (LLOQ) of 0.250 ng/mL. Further details of the bioanalytical method and its validation are provided in a subsequent section.

The PK analysis population included all subjects who received at least 1 dose of the investigational product and had at least 1 evaluable post-dose concentration value. PK parameters of lotilaner in whole blood were calculated by non-compartmental methods and summarized descriptively. The primary PK endpoints includes the following whole blood PK parameters for lotilaner: C_{max}, T_{max}, AUC (AUC_{0-12} and AUC_{0-24} for Day 1 and $AUC_{0-\tau}$ and AUC_{0-24} for Day 42), C_{trough} and for Day 24 AUC_{0-t}, T_{half}. Additionally, statistical analysis of steady state using natural-log (ln)-transformed C_{trough} values were performed and an analysis of

variance (ANOVA) model, with fixed day effect (repeated) was performed on the last three C_{trough} time points to determine the achievement of steady-state.

4.1.2 Pharmacokinetic Results

All participants (n=24) completed Study TRS-012 and included in the PK analysis. Table 4.1-1 summarizes the primary PK endpoints of lotilaner. The mean concentration versus time curve of lotilaner after single dose in healthy subjects is shown in the semi-log scale in Figure 4.1-1.

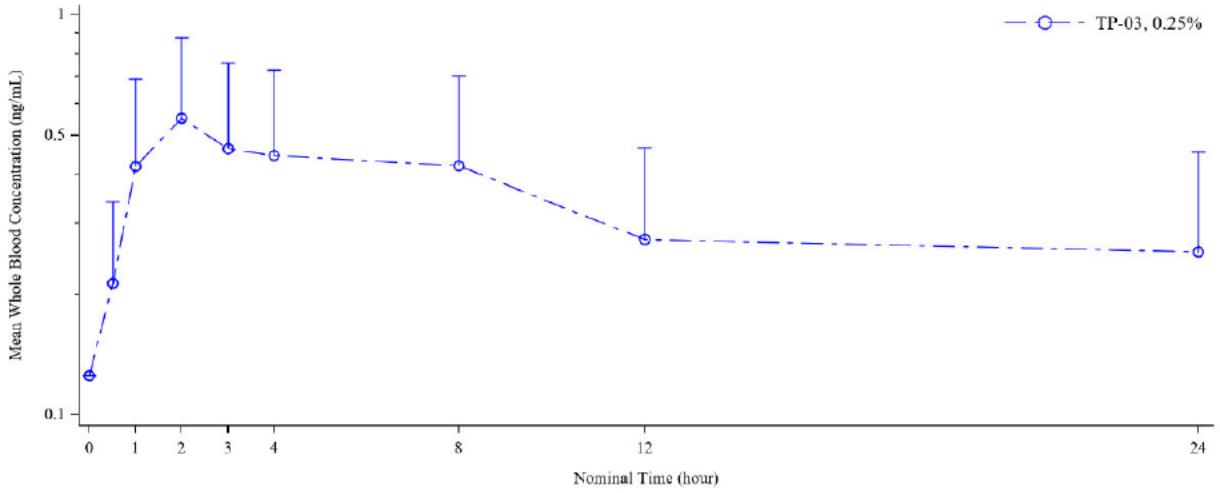
It is noted that all subjects exhibited quantifiable whole blood concentration at the last nominal time point, 2688h (112 days) after the last ophthalmic administration on Day 42. Figure 4.1-2 presents the PK profile of lotilaner after twice daily dosing into eye for 42 days. Figure 4.1-3 presents the variability of C_{trough} over study duration, 42 days.

Table 4.1-1 Summary Statistics of Whole Blood Lotilaner Pharmacokinetic Parameters Data Following Single and Repeat Topical Ocular Dose Administration of TP-03, 0.25% in Healthy Subjects

	T_{max} (hour) N= 24	C_{max} (ng/mL) N = 24	$AUC_{0-\tau}$[a] (hr*ng/mL) N= 24	AUC_{0-24} (hr*ng/mL) N= 24	AUC_{0-t} (hr*ng/mL) N = 24	T_{half} (hour) N= 21	$T_{half,eff}$ (hour) N= 18
Day 1	2.00 (1.00-8.05)	0.596 (51.7%)	5.75[b] (48.5%)	9.98[c] (48.8%)	6.44 (95.7%)	NC	NC
Day 42	1.00 (0.00-334)	17.8 (53.4%)	149 (52.2%)	293 (53.6%)	20600 (55.2%)	1400 (70.5%)	264 (46.6%)
R_{acc}	NC	33.4 (N=24) (62.0%)	32.3 (N=18) (45.9%)	36.8 (N=17) (49.8%)	NC	NC	NC

Source: Study Report TRS-012 Table 11-2
All parameters are reported as arithmetic mean (CV%), except T_{max}, which is reported as median (range).
Abbreviation: NC=not calculated, R_{acc}=accumulation ratio
[a]$AUC_{0-\tau}$ corresponds to the AUC_{0-12} for Day 1 where τ is the dosing interval of 12 hours; [b]N=18; [c] N=17

Figure 4.1-1 Mean (SD) Concentration-Time Profiles of Whole Blood Lotilaner Following Single Topical Ocular Dose Administration of TP-03, 0.25% in Healthy Subjects, Day 1 – Semi-Logarithmic Scale (PK Analysis Population).



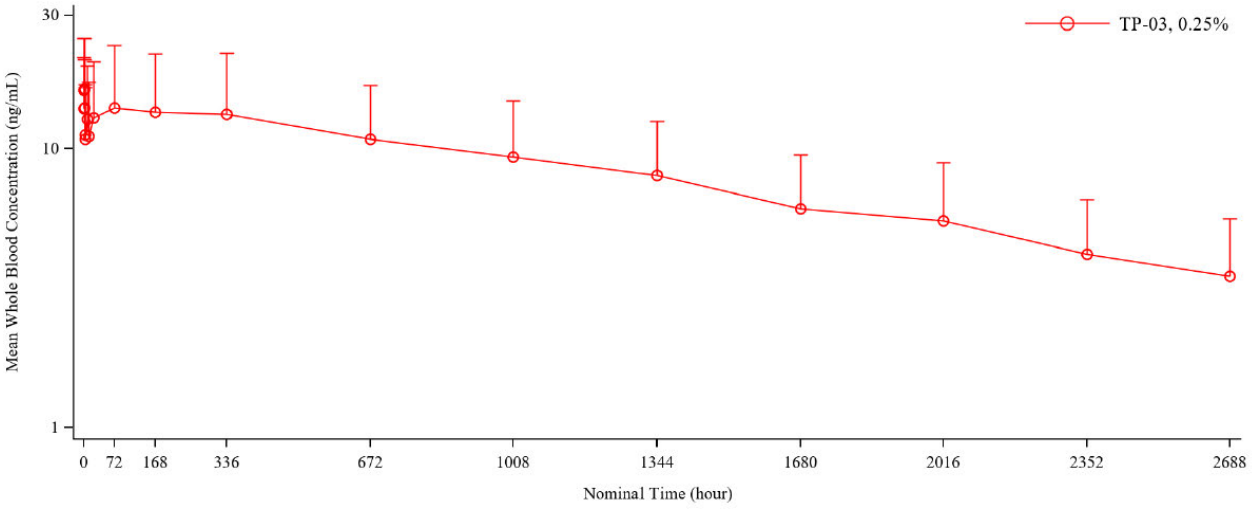
Source: Study Report of TRS-012, Figure 14-2
Concentration is taken as lower limit of quantification (LLOQ, 0.25 ng/mL)/2 if the concentration value is below limit of quantification (BLQ).

Following a single topical ocular administration of TP-03 0.25%, the T_{max} were reached within a median of 2.0 hours and 1.0 hour for Days 1 and 42, respectively. The median lotilaner whole blood T_{lag} was 0.500 hour indicating a delayed absorption after topical administration.
The peak (C_{max}) and total (AUC_{0-12}) exposure of lotilaner in whole blood increased after repeated ocular administration for 42 days. C_{max}, AUC_{0-12}, and AUC_{0-24} increased from 0.594 to 17.8 ng/mL, from 5.75 to 149 ng·h/mL, and from 9.98 to 293 ng·h/mL, respectively. The accumulation of lotilaner in whole blood was observed over the dosing interval with estimated accumulation ratios (R_{acc}) for C_{max}, AUC_{0-12}, and AUC_{0-24} of 33.4, 32.3, and 36.8, respectively (Table 3.2-1).

After 42 days of repeated ophthalmic administration, the estimated terminal phase half-life (T_{half}) was 1400 hours (58.3 days). However, the effective half-life ($T_{half,eff}$) which is based on the accumulation ratio over the dosing interval of 12 hours, was 264 h (11.0 days). This shorter effective half-life suggests a faster rate to steady state than the terminal phase half-life, which is supported by the mean C_{trough} data. The mean (SD) C_{trough} data on these days was 12.2 (8.08), 12.8 (8.58), 13.9, (7.31), and 11.1 (6.23) ng/mL for Days 35, 39, 42 and 42.5, respectively. An ANOVA model with fixed day effect using the last three C_{trough} time points at Days 39, 42, and 42.5 ng/mL was performed and resulted in a F-statistic of 14.9 and a p-value of <0.0001, suggesting that lotilaner had not reached systemic steady-state.

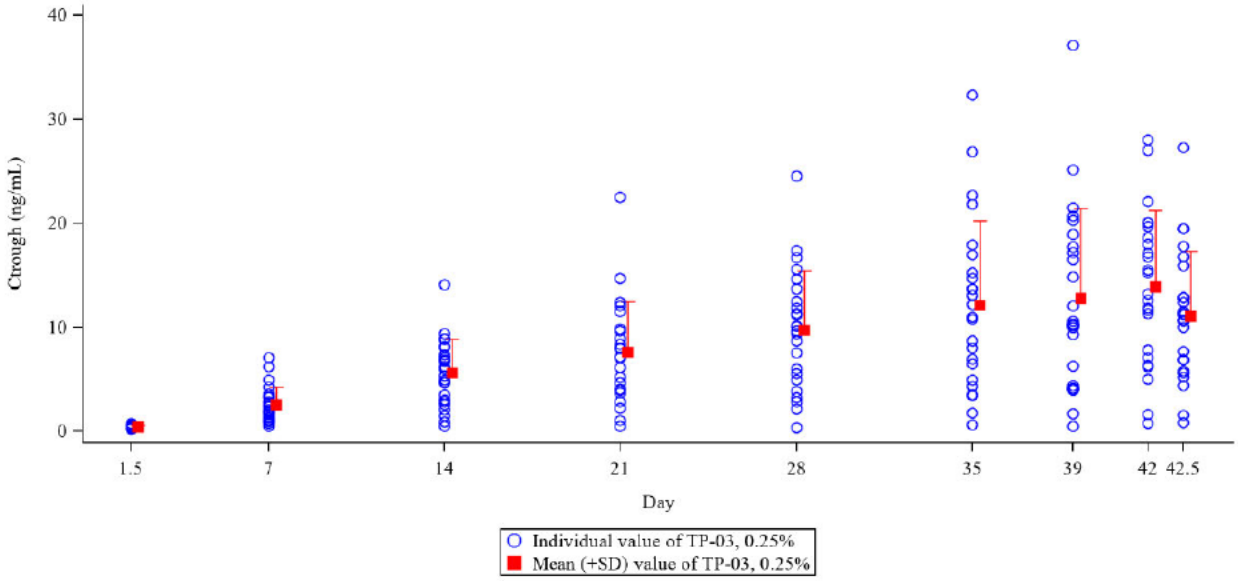
Overall, 106 of the 421 subjects (25.2%) experienced a total of 154 TEAEs (serious and nonserious combined). Of the 154 reported TEAEs, 115 (74.7%) were ocular and 39(25.3%) were non-ocular. The percentage of subjects who experienced TEAEs was comparable between TP-03 group (23.6%) and vehicle group (26.8%). For details in lotilaner safety profile, we defer to the Clinical Review.

Figure 4.1-2 Arithmetic Mean (+SD) Concentration-Time Profiles of Whole Blood Lotilaner Following Repeat Topical Ocular Dose Administration of TP-03, 0.25% in Healthy Subjects, Day 42 – Semi-Logarithmic Scale (PK Concentration Population)



Source: Study Report of TRS-012, Figure 14-4
Concentration is taken as LLOQ/2 if the concentration value is BLQ.

Figure 4.1-3 Scatter Plot of Whole Blood Lotilaner Trough Concentrations Following Repeat Topical Ocular Dose Administration of TP-03, 0.25% in Healthy Subjects (PK Analysis Population)



Source: Study Report of TRS-012, Figure 14-5
Day 1.5 and Day 42.5 represent the concentration measured 12 hours post-dose on Days 1 and 42, respectively.

4.1.3 Summary of Bioanalytical Method Validation and Bioanalytical Report

Bioanalytical Method Validation

The Applicant submitted one validation report for the bioanalytical method used in Study TRS-012 entitled "Validation of a Method for the Determination of Lotilaner in Human Whole Blood by HPLC with MS/MS Detection" (report number 8449-527). Based on this report, this method for analysis of lotilaner in human whole blood has been adequately validated.

Lotilaner concentrations in K_2EDTA human whole blood were determined using an HPLC/MS/MS assay with a validated quantitation range between 0.250 and 500 ng/mL. The method relies on protein precipitation to extract lotilaner (analyte) and (b) (4) (internal standard) from 0.250 mL human whole blood. Samples were quantified by obtaining the peak areas and determining the peak area ratio of analyte to internal standard.

Table 4.1-2 Bioanalytical method assessment

Information Requested	Data
Bioanalytical method validation report location	\\CDSESUB1\evsprod\NDA217603\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\8449-527\8449-527.pdf
Study name	Validation of a Method for the Determination of Lotilaner in Human Whole Blood by HPLC with MS/MS Detection

Assay parameters	
Analyte	Lotilaner, Lot 1950J013
Internal Standard (IS)	(b) (4), Lot 01
Quantitation range	0.250 (LLOQ) to 500 (ULOQ) ng/mL
QC levels	0.250, 0.750. 12.5, 200 and 400 ng/mL lotilaner
Type of assay	Protein precipitation / HPLC with MS/MS
Ionization mode, source type	Negative, electrospray ionization
Regression model	Linear
Weighting	1/x2
Standards	
Between-run precision	≤ 7.4%
Between-run accuracy	-2.3% to 1.3%
Precision and accuracy QCs	
Within-run precision (LLOQ)	≤ 9.9%
Within-run accuracy (LLOQ)	-14.8 to 8.4%
Within-run precision (low, mid, high)	≤ 9.0%
Within-run accuracy (low, mid, high)	-5.2% to 8.5%
Between-run precision (LLOQ)	12.5%
Between-run accuracy (LLOQ)	-4.0%
Between-run precision (low, mid, high)	≤ 6.9%
Between-run accuracy (low, mid, high)	-2.3% to 2.3%
Run-qualifying QCs	
Between-run precision	≤ 9.0%
Between-run accuracy	-7.0% to 5.0%
Analyte recovery (%CV)	≤ 8.6%
ISTD recovery (%CV)	≤ 6.6%
Selectivity	
Mean matrix factor	1.34
Precision (low)	3.3%
Precision (high)	2.4%
LLOQ QC	
Precision	11.0%
Accuracy	-1.2%
Matrix blanks	No interference
Over-range	4000 ng/mL at 10×, 8000 ng/mL at 20× dilution factors
Stability – matrix	
Short-term (ambient)	24h[a]
Postpreparative (2°C to 8°C)	499h[b]
Freeze/thaw (-20°C)	4 cycles[a]
Freeze/thaw (-70°C)	4 cycles[a]
Long-term (-10°C to -30°C)	28d[a]

Long-term (-60°C to -80°C)	315d[a]
Stability – stock solutions	
Bench-top stock (ambient)	100,000 ng/mL in DMF – 9h
Bench-top ISTD (ambient)	25.0 ng/mL in MeCN – 8h, 500.0 ng/mL in MeCN – 6h
Short-term stock (2°C to 8°C)	1.00 mg/mL in DMF – 42d, 5.00 ng/mL in DMF – 6h
Short-term ISTD (2°C to 8°C)	Not determined

d = days; DMF = dimethylformamide; h = hours; HPLC = high performance liquid chromatography; ISTD = Internal Standard; LLOQ = lower limit of quantitation; MeCN = acetonitrile; MS/MS = tandem mass spectrometry; QC = quality control; ULOQ = upper limit of quantitation
a Lotilaner concentrations of 0.750 and 400 ng/mL.
b Lotilaner concentrations of 0.750, 12.5, 200, and 400 ng/mL.

Bioanalytical Report Summary

The Applicant submitted one bioanalytical report detailing the performance of the PK sample analysis method used in Study TRS-012 entitled "Pharmacokinetic Study to Evaluate the Whole Blood Pharmacokinetics of TP-03 Following Six Week Topical Ocular Administration" (protocol number TRS-012). Based on this report, the PK assay performed with acceptable precision and accuracy in Study TRS-012.

Overall, in PK study TRS-012, 816 samples were analyzed. Samples were received between 10 May 2021 through 26 August 2021. Samples were analyzed between 09 March 2021 and 09 December 2021. All samples were kept at -60 to -80 °C. The total duration of sample storage was 275 days and therefore falls within the validated time frame for long-term stability at -60 - -80 °C (315 days).

Eight-point calibration curves (linear regression; $1/X^2$ weighting) with concentrations ranging from 0.250 to 500 ng/mL were run in duplicate with each sample analysis run. Across all concentrations in all sample runs, the percent coefficient of variation (%CV) and absolute value of the percent bias (|% Bias|) were ≤ 7.2% and ≤ 3.6%, respectively.

QCs at concentrations of 0.750 ng/mL (LQC), 12.5 ng/mL (LMQC), 200 ng/mL (MQC), and 400 ng/mL (HQC) were run in duplicate with each sample analysis run. One MQC sample in a single run was found to be outside the acceptance criteria with a calculated concentration that was close to HQC, 400 ng/mL. This sample was deactivated as an outlier. Across all QC values in all sample runs, excluding that value falling outside the acceptance range, the %CV and |% Bias| were ≤ 6.0% and ≤ 3.2%, respectively.

Incurred sample reanalysis (ISR) of 85 samples (approximately 10% of the total samples analyzed) yielded a passing rate of 88.2% (75/85) based on a maximum permitted deviation of 20% of the mean of the reference and repeat values. The Applicant's ISR evaluation is acceptable.

4.2 Phase 3 Trials: Studies TRS-009 and TRS-010

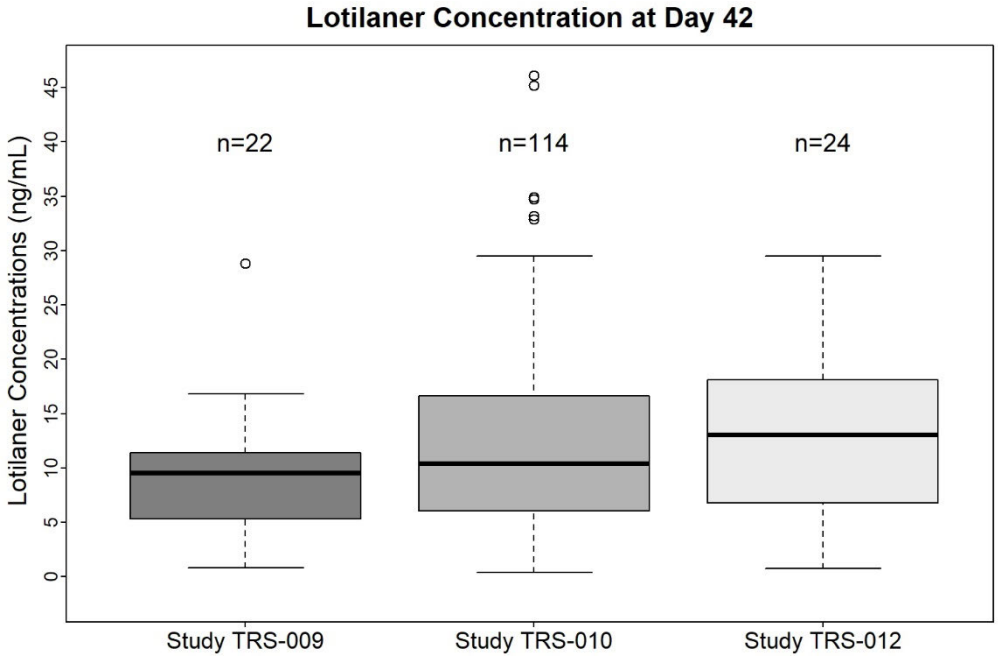
The Applicant conducted two randomized, controlled, multicenter, double-masked, parallel, phase 2b/3 and phase 3 trials patients with Demodex blepharitis: Studies TRS-009 and TRS-010. The participants were randomized to receive either lotilaner ophthalmic solution, 0.25% (TP-03,

0.25%) or matching placebo for 42 consecutive days. A single drop of TP-03, 0.25% ophthalmic solution was instilled in each eye twice daily (morning and evening) for 43 days.

In study TRS-009, blood samples were collected from participants at one study center (n=41) at the end of treatment to determine the concentration of lotilaner in whole blood. The mean (SD) lotilaner concentration in 22 subjects who received active treatment (TP-03) was 9.41 (5.99) ng/mL with range of 0.838 - 28.8 ng/mL. In study TRS-010, 412 patients with blepharitis were randomized (1:1) to receive either TP-03 (n=203) or vehicle (n=209). The mean (SD) lotilaner concentration was 12.5 (9.17) ng/mL with range of 0.381 – 46.1 ng/mL.

A box plot of the concentrations from studies TRS-009 and TRS-010 in comparison to those from Study TRS-012 is presented in Figure 4.2-1. For studies TRS-009 and TRS-010, the concentration was based on samples collected within 24 hours after the last administration of study drug. For Study TRS-012, the box plot represents the concentrations observed at 24 hours after the last administration of study drug on Day 43. The systemic exposure at the end of treatment in subjects with Demodex blepharitis was comparable with that in healthy subjects as presented in Figure 4.2-1.

Figure 4.2-1 Box Plot of Lotilaner Blood Concentrations 24 hours After the Last Dose in Studies TRS-009 and TRS-010 in Comparison to the Concentrations in Study TRS-012



Source: Reviewer's analysis using pc.xpt from Studies TRS-090, TRS-010, and TRS-012

4.2.1 Study TRS-009

Study Title: "Randomized, controlled, multicenter, double-masked, parallel, phase 2b/3 trial to evaluate the safety and efficacy of TP-03 for the treatment of demodex blepharitis (Saturn-1)"

To evaluate the safety and efficacy of TP-03 (lotilaner), the Applicant conducted a randomized, controlled, multicenter, double-masked, parallel, phase 2b/3 study in patients with *Demodex* blepharitis. The primary objective of this study was to demonstrate the safety and efficacy of TP-03 as a cure for *Demodex* blepharitis.

Four hundred twenty-one (421) patients with blepharitis were randomized (1:1) to receive either TP-03 (n=212) or vehicle (n=209). A single drop of the TP-03 was instilled in each eye twice daily (morning and evening) for 43 days. Study center staff supervised each subject's initial instillation of study drug to ensure proper technique.

Study TRS-009 enrolled adults (≥ 18 years) with each of the following in at least 1 eye: > 10 lashes with collarettes present on the upper eyelid (collarette score ≥ 2); at least mild erythema of the upper eyelid margin; and a Demodex density, upper and lower eyelids combined, of ≥ 1.5 mites per lash.

Sample Collection and Pharmacokinetic Assessments

As part of the Phase 2b/3 study, TRS-009 (Saturn-1), blood samples were collected from 41 subjects at the end of treatment to determine the concentration of lotilaner in whole blood. Subjects were requested to come in on Day 42, without controlling for when the last eye drop was administered to understand the general variability of systemic exposure in subjects after treatment. A summary of the study design is presented inTable 4.2-1.

Table 4.2-1 Study design of Study TRS-009

Subjects	Subjects with *Demodex* blepharitis
Method of administration	Topical, ocular
Study period	43 days
Name of active ingredient	Lotilaner
Investigational product	Lotilaner ophthalmic solution, 0.25% (2.5 mg/mL) (TP-03)
Batch number	182130 (LTL-001)
Dosing	Twice daily
Examination items	Human whole blood systemic exposure
Analytical assay	LOTIHBP 8449-527 - Protein precipitation / HPLC with MS/MS

HPLC = high performance liquid chromatography; MS/MS = tandem mass spectrometry

Summary of Bioanalytical Method Validation and Bioanalytical Report

Bioanalytical Method Validation

The bioanalytical method that had used in the Study TRS-009 was the identical analytical method used for Study TRS-012 (report number 8449-527).

Bioanalytical Report Summary

The Applicant submitted a bioanalytical report detailing the performance of the PK sample analysis method used in Study TRS-009. Based on this report, the PK assay performed with acceptable precision and accuracy in Study TRS-009.

Overall, in PK study TRS-009, 41 samples were analyzed. Samples were collected between 21 December 2020 through 29 December 2020. Samples were analyzed between 16 February 2021

and 19 February 2021. All samples were kept at -60 to -80 °C. The total duration of sample storage was 60 days and therefore falls within the validated time frame for long-term stability at -60 - -80 °C (315 days).

Eight-point calibration curves (linear regression; $1/X^2$ weighting) with concentrations ranging from 0.250 to 500 ng/mL were run in duplicate with each sample analysis run. Across all concentrations in all sample runs, the percent coefficient of variation (%CV) and absolute value of the percent bias (|% Bias|) were ≤ 9.2% and ≤ 5.2%, respectively.

QCs at concentrations of 0.750 ng/mL (LQC), 12.5 ng/mL (LMQC), 200 ng/mL (MQC), and 400 ng/mL (HQC) were run in duplicate with each sample analysis run. Across all QC values in all sample runs the %CV and |% Bias| were ≤ 5.2% and ≤ 8.8%, respectively.

Incurred sample reanalysis (ISR) of 22 samples (53.7% of the total samples analyzed) yielded a passing rate of 100% (22/22) based on a maximum permitted deviation of 20% of the mean of the reference and repeat values. The Applicant's ISR evaluation is acceptable.

4.2.2 Study TRS-010

Study Title: "Randomized, Controlled, Multicenter, Double-Masked, Parallel, Phase 3 Trial to Evaluate the Safety and Efficacy of TP-03 for the Treatment of Demodex Blepharitis (Saturn-2)"

To evaluate the safety and efficacy of TP-03 (lotilaner), the Applicant conducted a randomized, controlled, multicenter, double-masked, parallel group phase 3 study in patients with *Demodex* blepharitis. The primary objective of this study was to demonstrate the safety and efficacy of TP-03 as a cure for *Demodex* blepharitis.

Four hundred twelve (412) patients with blepharitis were randomized (1:1) to receive either TP-03 (n=203) or vehicle (n=209). A single drop of the TP-03 was instilled in each eye twice daily (morning and evening) for 43 days. Study center staff supervised each subject's initial instillation of study drug to ensure proper technique.

Study TRS-010 enrolled adults (≥ 18 years) with each of the following in at least 1 eye: > 10 lashes with collarettes present on the upper eyelid (collarette score ≥ 2); at least mild erythema of the upper eyelid margin; and a Demodex density, upper and lower eyelids combined, of ≥ 1.5 mites per lash.

Sample Collection and Pharmacokinetic Assessments

As part of the Phase 3 study, TRS-010 (Saturn-2), blood samples were collected from 327 subjects at the end of treatment to determine the concentration of lotilaner in whole blood. Subjects were requested to come in on Day 42, without controlling for when the last eye drop was administered to understand the general variability of systemic exposure in subjects after treatment. A summary of the study design is presented in Table 4.3-1.

Table 4.2-2 Study design of Study TRS-010

Subjects	Subjects with *Demodex* blepharitis

Method of administration	Topical, ocular
Study period	43 days
Name of active ingredient	Lotilaner
Investigational product	Lotilaner ophthalmic solution, 0.25% (2.5 mg/mL) (TP-03)
Batch number	182130 (LTL-001)
Dosing	Twice daily
Examination items	Human whole blood systemic exposure
Analytical assay	LOTIHBP 8449-527 - Protein precipitation / HPLC with MS/MS

HPLC = high performance liquid chromatography; MS/MS = tandem mass spectrometry

There were 203 subjects administered TP-03 and 114 had a blood sample collected that could be analyzed for lotilaner. The mean (SD) lotilaner concentration was 12.5 (9.17) ng/mL with range of 0.381 – 46.1 ng/mL. Two subjects in the placebo group presented with reportable values. One of the subjects had multiple dogs in the household that were on the flea and tick medication, Credelio, which has the same active ingredient (lotilaner) as the TP-03. The subject may have absorbed the drug through the skin while handling it monthly or also through contact with the dogs. The other subject was in a household where there were 2 study participants, 1 on vehicle and 1 on TP-03. The subject believes the medications could have been mixed up once.

Summary of Bioanalytical Method Validation and Bioanalytical Report

Bioanalytical Method Validation

The bioanalytical method that had used in the Study TRS-010 was the identical analytical method used for Study TRS-012 (report number 8449-527).

Bioanalytical Report Summary

The Applicant submitted a bioanalytical report detailing the performance of the PK sample analysis method used in Study TRS-010. Based on this report, the PK assay performed with acceptable precision and accuracy in Study TRS-010.

Overall, in PK study TRS-010, 311 samples were analyzed. Samples were collected between 8 June 2021 through 14 March 2022. Samples were analyzed between 15 December 2021 and 06 April 2022. All samples were kept at -60 to -80 °C. The total duration of sample storage was 302 days and therefore falls within the validated time frame for long-term stability at -60 - -80 °C (315 days).

Eight-point calibration curves (linear regression; $1/X^2$ weighting) with concentrations ranging from 0.250 to 500 ng/mL were run in duplicate with each sample analysis run. Across all concentrations in all sample runs, the percent coefficient of variation (%CV) and absolute value of the percent bias (|% Bias|) were ≤ 7.3% and ≤ 5.0%, respectively.

QCs at concentrations of 0.750 ng/mL (LQC), 12.5 ng/mL (LMQC), 200 ng/mL (MQC), and 400 ng/mL (HQC) were run in duplicate with each sample analysis run. Across all QC values in all sample runs the %CV and |% Bias| were ≤ 8.7% and ≤ 7.5%, respectively.

Incurred sample reanalysis (ISR) of 26 samples (8.4% of the total samples analyzed) yielded a passing rate of 73% (19/26) based on a maximum permitted deviation of 20% of the mean of the reference and repeat values. The Applicant's ISR evaluation is acceptable.

4.3 *In vitro* Study Results

4.3.1 Study 8443928

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

Study Design

Test System	Mouse, Rat, Dog, and Human hepatic pooled blood and plasma
Method of Administration	In vitro
Conc. of Test Compound	Lotilaner at final conc. 5 µM incubated for 5 h at 37°C
Examination items	Ultracentrifugation, dialysis
Detection Method	HPLC
Compound / Batch Number	1821J001
GLP Compliance	No

Summary of Results:
Lotilaner plasma protein binding was high in mouse, rat, dog, and human, with unbound Lotilaner ≤ 0.1%. Actual mean values of percent unbound were 0.0129, 0.0221, 0.0730, and 0.0732 for lotilaner (5 µM) after dialysis in mouse, rat, dog, and human plasma, respectively. Lotilaner was stable in mouse, rat, dog, and human whole blood for 60 minutes after incubation at 37°C. The results indicated a non-concentration dependent, low, partitioning to blood cells for lotilaner across the species tested. Partitioning of lotilaner to rodent (mouse and rat) blood cells was <1%, human blood varied from donor to donor and was in the range of 0-20 % and generally approximately closer to 10%, while dog blood: blood partitioning was highest at ≤ 22.1%.

4.3.2 Study 8445531

Study title: "Metabolism of Lotilaner in Mouse, Rat, Rabbit, Dog, Monkey, and Human Primary Hepatocytes"

Study Design

Test System	Mouse, rat, rabbit, dog, monkey, and human primary hepatocytes
Method of Administration	In vitro
Conc. of Test Compound	0.5 and 5 µM Lotilaner
Examination items	CYP enzymes, glucuronidation, and sulfation
Detection Method	LC-MS
Compound / Batch Number	1821J001
GLP Compliance	No

Summary of Results:

Lotilaner (0.5 and 5 µM) was incubated with mouse, rat, rabbit, dog, monkey, and human hepatocytes for 0, 30, 60, 90, 120, and 240 minutes. There was no significant difference in degradation between 0.5 and 5 µM lotilaner. The amount of 5 µM lotilaner remaining after incubation in hepatocytes for 4 h in mouse was 86.6-93.8%, in rat 80.2-95.0%, in rabbit 95.4-104%, in dog 88.0-105%, in monkey 93.3-99.9% and in human hepatocytes 96.3-105%. All concentration changes observed in hepatocytes from all species tested were not time dependent. These results indicated that lotilaner was metabolically stable in hepatocytes from mouse, rat, rabbit, dog, monkey, and human under the experimental conditions of the study.

4.3.3 Study 8445547

Study Title: "Identification of Human Drug Metabolizing Enzymes Involved in the Metabolism of Lotilaner"

Study Design

Test System	Human liver microsomes
Method of Administration	In vitro
Conc. of Test Compound	1 µM Lotilaner
Examination items	HLM, S9 fraction, and cytosol
Detection Method	LC-MS
Compound / Batch Number	1821J001
GLP Compliance	No

Summary of Results:
The study was designed to identify the human drug metabolizing enzymes involved in the metabolism of lotilaner. The results of this pilot study indicated limited or no metabolism of lotilaner. The results also indicated the potential for non-specific binding to plasticware, and that lotilaner was chemically stable under the conditions of the study. Lotilaner does not appear to be metabolized by typical enzymes associated with xenobiotic metabolism.

4.3.4 Study 8445532

Study Title: "Evaluation of Cytochrome P450 Induction Following Exposure of Primary Cultures of Human Hepatocytes to Lotilaner"

Study Design

Test System	Human hepatocytes
Method of Administration	In vitro
Conc. of Test Compound	Lotilaner
Examination items	CYP1A2, CYP2B6, and CYP3A4
Detection Method	LC-MS/MS
Compound / Batch Number	1821J001
GLP Compliance	No

Summary of Results:

Lotilaner did not induce enzymatic activity of CYP2B6, CYP3A4/5, nor enzymatic activity and mRNA levels for CYP1A2. It did induce mRNA>2-fold at average concentrations of 3 µM for CYP2B6 and 3.5 µM for CYP3A4. The mRNA fold increase for these two enzymes was moderate and the levels of fold induction were below the positive control. The induction potential for lotilaner is low.

4.3.5 Study 8445533

Study Title: "Inhibitory Potential of Lotilaner towards Human Liver Microsomal Cytochrome P450 Enzymes"

Study Design

Test System	Human liver microsomes
Method of Administration	In vitro
Conc. of Test Compound	Lotilaner at final conc. 5 µM incubated for 5 h at 37°C
Examination items	CYP1A2, CYP2B6, CYP2C8, CYP2C19, CYP2D6, and CYP3A4/5
Detection Method	LC-MS
Compound/ Batch Number	1821J001
GLP Compliance	No

Summary of Results:
When tested at concentrations up to 5 µM, lotilaner showed direct inhibition of CYP2C8, CYP2C9, CYP2C19, and CYP2D6. The IC_{50} values (uncorrected for microsomal protein binding) for direct inhibition were listed in Table 4.4-1. Lotilaner did not inhibit CYP1A2, CYP2B6, CYP3A4/5 (testosterone or midazolam substrates) up to the highest concentration tested of 5 µM. Additionally, lotilaner did not exhibit metabolism-dependent inhibition of any CYP enzymes tested. In additional work, the inhibition constant (K_i) for CYP2C8 was determined to be 0.298 µM.
Lotilaner was tested for microsomal protein binding and was found to be highly bound to HLM, with the mean percent unbound ranging from 0.206 to 1.19%. Based on HLM protein binding, corrected IC_{50} and K_i values were determined. The $K_{i,u}$ (K_i unbound) value for CYP2C8 was 0.402 µM as shown in Table 4.4-1. It is anticipated that the $K_{i,u}$ for the other enzymes will be approximately > 3µM based on the CYP2C8 data. Of note is the unbound maximum observed concentration of any one subject in the clinical study TRS-012 is 0.41 ng/mL (0.00069 µM), conservatively assuming 1% free drug (protein binding is 99.9% bound) at the highest observed C_{max} in any one subject of 41 ng/mL.

Table 4.3-1 Human Liver CYP Isozyme Inhibition

Enzyme	Direct Inhibition			Metabolism-Dependent Inhibition Conclusion
	Inhibitor Conclusion	Total IC_{50} (K_i) (µM)[a]	$IC_{50,u}$ ($K_{i,u}$) (µM)	
CYP1A2	No	NA	NA	No
CYP2B6	Yes	>5	NA	No
CYP2C8	Yes	0.402	0.00383	No
CYP2C9	Yes	3.72	0.0294	No

CYP2C19	Yes	1.56	0.0134	No
CYP2D6	Yes	4.70	0.0403	No
CYP3A4/5[b]	No	NA	NA	No
CYP3A4/5[c]	No	NA	NA	No

Source: Section 2.6.4 Pharmacokinetics-Written-Summary Table 19; IC_{50}=Concentration for 50% inhibition

4.3.6 Study 5445534

Study Title: "Evaluation of Lotilaner as a Substrate and/or Inhibitor of a Panel of Human Drug Transporters"

Study Design

Test System	Human embryonic kidney (HEK) 293 cells, Caco-2 subclone C2BBe1, membrane vesicles prepared from baculovirus infected cells (Sf9) expressing BSEP protein
Method of Administration	In vitro
Conc. of Test Compound	0.5 and 5 µM Lotilaner
Examination items	OAT3, OCT1, OCT2, OATP1B1, OATP1B3, P-gp, BCRP, BSEP
Detection Method	LC-MS
Compound/ Batch Number	1821J001
GLP Compliance	No

Summary of Results:
Uptake of lotilaner (0.5 and 5 µM) by human transporters OCT1, OATP1B1 and OATP1B3 was ≤1.14-fold for all transporters tested at both lotilaner concentrations compared to vehicle control. These results indicated lotilaner was not a substrate of any of the transporters tested. Uptake of probe substrates by transporters OAT3, OCT2, OATP1B1, and OATP1B3 was conducted in the absence and presence of lotilaner (0.5 and 5 µM) or a known inhibitor.
Lotilaner exhibited <50% inhibition of OATP1B1 at 5 µM, and no inhibition of OAT3, OCT2, and OATP1B3 SLC transporters. The observed inhibition of OATP1B1 at the concentrations evaluated was not sufficient to determine the IC_{50} and thus the value was estimated to be >5 µM.
Lotilaner (0.5 and 5 µM) was evaluated as a substrate for P-gp and BCRP and the efflux ratio was ≤1.67 for all the efflux transporters at both lotilaner concentrations, thus the results indicated lotilaner was not a substrate of these ABC efflux transporters. Lotilaner (0.5 and 5 µM) was evaluated as an inhibitor for P-gp and BCRP in the same experimental systems and was found to be an inhibitor of BCRP, but not P-gp. The maximal inhibition against BCRP was <50% at 5 µM, the highest concentration evaluated, and there was insufficient data to determine the IC_{50} value, thus the value was estimated to be >5 µM.
Finally, the ATP-dependent uptake of lotilaner (0.5 and 5 µM) by BSEP membrane vesicles was not appreciably different in ATP- or AMP-containing sample, with a signal-to-noise ratio ≤1.17, indicating that lotilaner was not a substrate of BSEP.

4.3.7 Study TRX-20-01

Study title: "In Vitro Effect of Lotilaner on hERG Current (IKr) Expressed in Human Embryonic Kidney (HEK) Cells"

Study Design

Test System	Human embryonic kidney; transformed with adenovirus and transfected with human-ether-ago-go cDNA
Method of Administration	In vitro
Conc. of Test Compound	0.1, 1, 10, 30, and 100 μM Lotilaner
Examination items	hERG current
Detection Method	Whole-cell variant of the patch clamp method
Compound/ Batch Number	1821J001
GLP Compliance	No

Summary of Results:
The effects of lotilaner (0.1 μM to 100 μM, nominal) on hERG current amplitude at physiologic temperatures were characterized. Lotilaner had little effect on hERG current amplitude with 100μM blocking hERG current by 8.7 ± 1.0%.

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