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APPLICATION NUMBER:

218158Orig1s000

CLINICAL PHARMACOLOGY REVIEW(S)

CLINICAL PHARMACOLOGY MEMO

NDA Number	218158
Link to EDR	\\CDSESUB1\evsprod\NDA218158\0020
Applicant	Formosa Pharmaceuticals Inc.
Brand Name	To-be-determined
Generic Name	Clobetasol Propionate
Dosage Form and Strength	Ophthalmic nanosuspension, 0.05%
Submission Type	Standard
Submission Date	05/04/2023
PUDFA Goal Date	03/04/2024
Proposed Indication	Treatment of post-operative inflammation and pain in patients following ocular surgery
Proposed Dosing Regimen & Instructions	One drop in the (b) (4) operated eye twice daily for 14 days
Associated IND	128133
OCP Division	Division of Inflammation and Immune Pharmacology (DIIP)
OCP Review Team	Hyewon Kim, Ph.D. Clinical Pharmacology Reviewer, DIIP Ping Ji, Ph.D. Team Leader, DIIP

Introduction

NDA 218148 for APP13007 (clobetasol propionate ophthalmic suspension 0.05%) was submitted under Section 505(b)(2) of the Federal Food, Drug, and Cosmetic Act and 21 CRF 314.54 with Temovate ointment 0.05% (NDAs 019322, 019323, 019966, 020337) as a listed drug. The clinical pharmacology review of this NDA was completed and filed in DARRTS on January 10, 2024.

On February 22, 2024, the Agency requested information to justify reliance on FDA's finding of nonclinical safety for Temovate topical ointment by establishing a scientific bridge based on a comparison of the AUC and C_{max} for APP13007 established in the PK study, CPN-102, to the AUC and C_{max}, respectively, identified in the published literature for Temovate topical ointment (e.g., the 2008 publication by Kimball AB *et al.*).

On February 26, 2024, the Applicant submitted response to the information request. The Applicant compared the observed C_{max} and AUC_{0-12} of APP13007 from Study CPN-102 to those following Temovate administration in the literature, Kimball AB *et al.*, 2008.

This memo is to review the pharmacokinetic data in the Applicant's response to the information

request.

Data Submitted by the Applicant

Data source:

APP13007: An Open-Label, Sequential Dosing Study to Evaluate Systemic Drug Exposure following Ocular Instillation of 0.05% APP13007 in Healthy Subjects (Clinical Study Report CPN-102).

Temovate ointment 0.05%: Clobetasol Propionate Emulsion Formulation Foam 0.05%: Review of Phase II Open-Label and Phase III Randomized Controlled Trials in Steroid-Response Dermatoses in Adults and Adolescents. Kimball AB et. al., 2008, J Am Acad Dermatol 59(3):448-454.

PK exposure:

Table 1 Comparison of Summary Statistics of Cmax and AUCs of Clobetasol Propionate between APP13007 and the RLD (Temovate Ointment 0.05%)

	APP13007				TEMOVATE	
	Period 1 (First Dose)		Period 2 (Second Dose)		Day 8 (First Dose)	
Statistics	Cmax (pg/mL)	$AUC_{(0-24)}$ (hr*pg/mL)	Cmax (pg/mL)	$AUC_{(0-12)}$ (hr*pg/mL)	Cmax (pg/mL)	$AUC_{(0-12)}$ (hr*pg/mL)
N	12	12	10	10	16	16
Mean	32.2	56.9	36.5	77.2	188.1	1572.9
SD	43.6	90.1	59.8	143.5	274.2	2436.8
%CV	135.4	158.2	163.7	185.9	145.8	154.9
Min	0	0	0	0	0.0	0.0
Median	0	0	0	0	100.5	796.4
Max	128.0	273.1	182.0	441.6	1104.3	10133.1

Source: Sponsor's response to the information request dated February 26th, 2024.

Reviewer's Analysis

In Study CPN-102, there were four subjects who had at least two clobetasol propionate concentrations above lower limit of quantification (LLOQ, 0.04 ng/mL). PK samples were collected up to 24 hours post-dose. However, clobetasol propionate concentrations were all below LLOQ after 4 hours post-dose.

Table 2 Concentrations and AUC_{0-12} Following the First Dose of APP13007 0.05%

Time	0 hour	0.25 hour	0.5 hour	1 hour	1.5 hour	2 hour	3 hour	4 hour	AUC_{0-12}
(b) (6)	0	0	64.4	**128**	105	94.8	61.3	0	273.1
	0	0	0	60	68.8	58.5	42	0	150.3
	0	43.7	74.1	75.1	64.6	55	0	0	149.8
	0	0	0	62.3	51.7	44.6	0	0	90.5

Concentrations in pg/mL; AUC in pg·h/mL

Source: Reviewer's independent analysis using Table 14.2.1 in Clinical Study Report CPN 102

Table 3 Concentrations and AUC_{0-12} Following the Second Dose of APP13007 0.05%

Time	0 hour	0.25 hour	0.5 hour	1 hour	1.5 hour	2 hour	3 hour	4 hour	AUC_{0-12}
(b) (6)	0	0	82.5	**182**	171	134	80.3	53.4	441.6
	0	0	0	40.3	0	40.3	0	0	50.4
	0	0	60.4	87.5	81.4	69	44.7	0	203.6
	0	41.7	55.3	46.5	43.4	0	0	0	76.1

Concentrations in pg/mL; AUC in pg·h/mL
Source: Reviewer's independent analysis using Table 14.2.1 in Clinical Study Report CPN 102

The C_{max} and AUC_{0-12} of clobetasol propionate following one time and two times ocular administrations of APP13007 are lower than those following dermal application of Temovate® ointment 0.05% at a clinically relevant dose.

Reviewer's Conclusion:
A scientific "bridge" is acceptable between APP13007 and Temovate ointment 0.05%.

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/s/

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Office of Clinical Pharmacology Review

NDA Number	218158
Link to EDR	\\CDSESUB1\evsprod\NDA218158\0001
Submission Date	5/4/2023
Submission Type	505(b)(2) NDA; Standard
Brand Name	(b) (4)
Generic Name	Clobetasol propionate
Dosage Form and Regimen	Ophthalmic nanosuspension, 0.05% One drop in the (b) (4) operated eye twice daily for 2 weeks
Route of Administration	Topical ocular
Proposed Indication	Treatment of post-operative inflammation and pain in patients following ocular surgery
Applicant	Formosa Pharmaceuticals Inc.
Associated IND	IND 128133
OCP Review Team	Hyewon Kim, Ph.D. Ping Ji, Ph.D.
OND Division	Division of Ophthalmology
OCP Division	Division of Inflammation and Immune Pharmacology

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1 EXECUTIVE SUMMARY

The Applicant, Formosa Pharmaceuticals Inc., has submitted a 505(b)(2) for APP13007 (a clobetasol propionate ophthalmic nanosuspension 0.05%), a synthetic corticosteroid, for the treatment of post-operative inflammation and pain in adult patients following ocular surgery. The proposed dosing regimen is one drop in the (b) (4) operated eye twice daily (BID) for 2 weeks. TEMOVATE® 0.05% (NDAs 019322, 019323, 019966, and 020337) is used as the Reference Drug (RD) in this submission.

In this application, the Applicant completed one clinical PK phase 1 trial (CPN-102), one phase 2 dose-selection trial (CPN-201), and two pivotal phase 3 efficacy and safety trials of APP13007 (CPN-301 and CPN-302). No PK sample was collected in the phase 2 and phase 3 trials, CPN-201, CPN-301, and CPN-302. Therefore, this clinical pharmacology review focused on the phase 1 PK study.

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 218158 for the proposed clobetasol propionate ophthalmic nanosuspension 0.05% 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, double-masked, placebo-controlled phase 3 trials (Studies CPN-301 and CPN-302) in adult patients following ocular surgery. Phase 2 dose-ranging trial provides supportive evidence for its efficacy of APP13007 0.05%.
General dosing instructions	One drop of clobetasol propionate ophthalmic nanosuspension 0.05% into the affected eye twice daily beginning the day after surgery and continuing throughout the first 2 weeks of the post-operative period.
Dosing in patients (intrinsic and extrinsic factors)	No dose adjustment is recommended for patients based on intrinsic and extrinsic factors.
Labeling	See Section 2.2.4
Bridge between the to-be-marketed and clinical trial formulations	Not applicable. To-be-marketed formulation of APP13007 0.05% was used in the phase 1 PK, phase 2 dose-selection, and pivotal phase 3 trials.

1.2 Post Marketing Requirement

None

2 SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

The clinical pharmacology assessment included a review of the PK data from a single- and multiple-dose PK study in healthy subjects, Study CPN-102, its associated bioanalytical method as well as the dose-response findings from phase 2 dose-ranging trial, Study CPN-201.

2.1 Pharmacology and Clinical Pharmacokinetics

Clobetasol propionate is a synthetic corticosteroid that has anti-inflammatory, antipruritic, and vasoconstrictive properties. However, the mechanism of the anti-inflammatory activity of the topical steroids is unclear.

The PK study, Study CPN-102, showed systemic exposure of clobetasol propionate following ocular instillation of APP13007 was minimal in healthy subjects. Four out of, in total, 12 healthy subjects had more than 2 measurable plasma concentrations of clobetasol propionate after receiving APP13007 0.05% once or twice (12 hours apart) in the eye. After APP13007 0.05% dosing into the eye, C_{max} of clobetasol propionate ranged 0.040-0.182 ng/mL with T_{max} observed between 0.5 and 1 hour. Clobetasol propionate concentrations declined rapidly to be lower than LLOQ after 4 to 5 hours post-dose.

2.2 Dosing and Therapeutic Individualization

2.2.1 General Dosing

Proposed dosing regimen is one drop in the [redacted] (b) (4) operated eye twice daily (BID) for 2 weeks for the treatment of post-operative inflammation and pain in adult patients following ocular surgery. Proposed dose and dosing regimen were evaluated in two pivotal phase 3 trials that provided substantial evidence of efficacy and safety of clobetasol propionate arm compared to placebo arm. See clinical review for the design of phase 2a and phase 3 trials, the efficacy and safety results.

2.2.2 Therapeutic Individualization

Therapeutic individualization is not applicable for clobetasol propionate nanosuspension because it is locally administered with minimum systemic exposure.

2.2.3 Outstanding Issues

None.

2.2.4 Summary of Labeling Recommendations

Clinical Pharmacology team recommends the Applicant include the summary information of observed clobetasol concentrations in Section 12.3 Pharmacokinetics and revise Section 8.2 Lactation based on the available data.

3 COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

The proposed product is a clobetasol propionate ophthalmic nanosuspension 0.05% (b) (4) for the treatment of post-operative inflammation and pain in adult patients following ocular surgery. To date, FDA-approved clobetasol propionate formulations include aerosol (foam), cream, gel, lotion, ointment, shampoo, solution, and spray for topical administration. This NDA is the first application of ophthalmic formulation of clobetasol propionate indicated to administer to the operated eye. Proposed dosing regimen is one drop of clobetasol propionate 0.05% in the (b) (4) operated eye BID for 2 weeks.

The proposed product was evaluated under IND 128133. 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 128133, Pre-IND (Jan. 2016)	▪ The Agency agreed that the Applicant can rely on the Agency's efficacy and safety findings as described in FD&C Act Sec. 505(b)(2) by establishing an adequate bridge between APP13007 and listed drug(s).
End-of-Phase 2 (Sep. 2020)	▪ The Agency agreed with the proposed dose, 1 drop of 0.05% APP13007 administered twice daily for 14 days, was acceptable to be evaluated in the pivotal phase 3 trials. ▪ The Agency agreed that phase 1 clinical PK study was adequate to characterize the systemic exposure of APP13007 0.05% and no further clinical study was required.
Pre-NDA (Oct. 2022)	▪ The Agency agreed that the summary data from the four clinical studies are sufficient to support filing of an NDA for APP13007.

Source: Reviewer's summary based on meeting minutes located in DARRTS for IND 128133

3.2 General Pharmacology and Pharmacokinetic Characteristics

Pharmacology	**APP13007**	**TEMOVATE® or Literature**
Mechanism of Action	Clobetasol is a synthetic corticosteroid that has anti-inflammatory, antipruritic, and vasoconstrictive properties. The mechanism of the anti-inflammatory activity of the topical steroids is unclear.	
Active Moiety	Clobetasol propionate	
General Information		
Bioanalysis	The method for analysis of clobetasol propionate in human plasma has been adequately validated in report AP19-055-01.	Not available
Maximum Daily Dose	0.05 mg/day	3.57 mg/day

Absorption		
Maximum Concentration (C_{max}, ng/mL)	Following a single or double (12 hours apart) dosing of APP13007 0.05% one drop in the eye to 12 subjects, 8 subjects had no detectable concentrations at any timepoints, the rest 4 subjects had C_{max} as 0.182, 0.043, 0.087, and 0.055 ng/mL, respectively.	Range of C_{max} on Day 8 was 0.0-1.1 ng/mL with median of 0.1 ng/mL after 3.5 g twice daily Temovate® 0.05% dermal application.
Time to reach C_{max} (T_{max}, hours)	Range of T_{max} was 0.5 - 1 hour	Mean T_{max} was 5.3 hours (max of T_{max} = 12 hours)[1]
Accumulation	No accumulation was observed following two optic administrations with 12 hours apart.	Not available
Distribution		
Protein Binding	Not determined	
Metabolism		
Metabolic Pathway	Not determined	Corticosteroids are metabolized primarily in the liver[2]
Elimination		
Excretion Pathway	Not determined	Corticosteroids are excreted by the kidneys[2]

[1] Kimball *et al.*, J Am Acad Dermatol. 2008 Sep;59(3):448-54
[2] TEMOVATE® LABEL

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?

Clinical pharmacology information does not provide pivotal or supportive evidence of effectiveness of clobetasol propionate 0.05% ophthalmic nanosuspension. Since clobetasol propionate is administered as eye drop and the site of action is local (the eye), the systematic exposure is not expected to affect treatment effect by mechanism.

The recommended dosing regimen was evaluated in phase 2a dose-finding trial (CPN-201) and two phase 3 pivotal trials: Studies CPN-301 and CPN-302. Refer to the clinical and statistical reviews for more information on efficacy and safety assessments.

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

Yes. Proposed dose and dosing regimen was evaluated in two randomized placebo-controlled trials (CPN-301 and CPN-302) in patients following ocular surgery. The results in phase 3 trials demonstrated the efficacy and the safety of clobetasol propionate 0.05% for the treatment of

post-operative inflammation and pain in adult patients following ocular surgery. 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 clobetasol propionate ophthalmic nanosuspension 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. No drug-drug interaction study was conducted as the proposed product is locally administered as topical eye drop with low systemic exposure.

4 APPENDICES: INIDIVIDUAL STUDY REPORT

4.1 Phase 1 Trial: Study CPN-102

The Applicant conducted a single center, open-label, 2-period, sequential dosing study (Study CPN-102) in healthy subjects to evaluate the systemic exposure to clobetasol propionate based on plasma concentrations following ocular instillation of clobetasol propionate ophthalmic suspension (APP13007), 0.05%.

Study Title: "An Open-Label, Sequential Dosing Study to Evaluate Systemic Drug Exposure following Ocular Instillation of 0.05% APP13007 in Healthy Subjects"

Design: Study CPN-102 enrolled healthy males and females who are aged between 18 and 55 years and a body mass index (BMI) of 18.0 - 31.0 kg/m^2, inclusive. Subjects with clinically relevant abnormalities based on the medical history (including history of ophthalmological, cardiac, gastrointestinal, hepatic, renal, other organ diseases or abnormalities, including neurological or psychiatric illness), physical examination, or clinical laboratory evaluation were excluded from the enrollment. No prescription drugs including corticosteroid preparations, over the counter drugs including antacids, high-dose multivitamins, nutritional supplements, and herbal preparations were allowed from 14 days prior to the first study drug administration to the completion of the study. As-needed use of acetaminophen or nonsteroidal analgesics was permitted.
Participants received the first dose of study drug in the morning of Day 1 and were discharged in the morning of Day 2, Period 1. After a washout period of at least 6 days following dosing on Period 1, participants received one drop of APP13007 0.05% on the evening of Day -1 of Period 2. Approximately 12 hours after the first dose in Period 2, participants received the second drop of APP13007 0.05%. Participants were confined to the clinical site from the evening of Day -1 until 24 hours post-last dose in each period. The primary endpoint was plasma concentrations of clobetasol propionate following the first drop of 0.05% APP13007 and the second drop of 0.05% APP13007 simulating twice a day dosing schedule.

4.1.1 Sample Collection and Pharmacokinetic Assessments

Plasma samples for clobetasol concentration were collected within 15 minutes prior to dosing and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 20, and 24 hours post-dose on Day 1 of each period. Subjects were not fasted during the dosing and sampling periods but received light meals throughout the day (approximately 2000 kcal in a day). On Day 1 of each dose period, subjects received breakfast prior to dosing.

PK samples were analyzed using a validated high-performance liquid chromatography-mass spectrometry-mass spectrometry (HPLC/MS/MS) method to determine the concentration of clobetasol propionate in human plasma with a lower limit of quantitation (LLOQ) of 0.04 ng/mL. Further details of the bioanalytical method and its validation are provided in a subsequent section. All samples were collected using tubes containing K_2EDTA. Samples were stored at -70°C until they were shipped on dry ice to the bioanalytical laboratory.

4.1.2 Pharmacokinetic Results

All randomized subjects (n=12) completed Period 1, and 10 subjects completed both Period 1 and Period 2. The PK population included subjects who received at least 1 dose of clobetasol propionate ophthalmic nanosuspension (n=12).

Out of 12 subjects, there were 4 subjects in each dose period had more than 1 plasma sample containing clobetasol propionate concentrations above LLOQ in each period. Following an ocular administration of APP13007 0.05%, the time to reach a maximal concentration (T_{max}) ranged 0.5-1 hour as presented in Table 4.1-1. Overall, when measurable, the clobetasol propionate levels were low and ranged from 0.040 to 0.182 ng/mL, quickly decaying to below LLOQ at 4-5 hours post-dose. There was no evidence of accumulation in plasma following the second dose in Period 2 and the systemic bioavailability of clobetasol propionate is low.

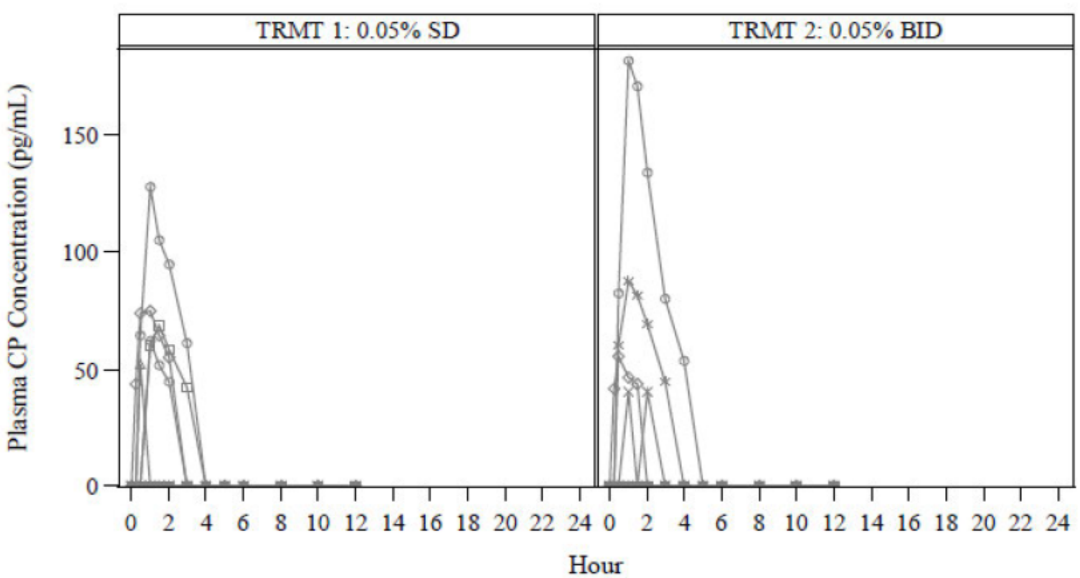
Table 4.1-1 Summary of Plasma Clobetasol Propionate Concentration (pg/mL)

Site ID	Subject/ Statistics	Pre-Dose	0.25 Hr	0.5 Hr	1 Hr	1.5 Hr	2 Hr	3 Hr	4 Hr	5 Hr	6 Hr	8 Hr	10 Hr	12 Hr	16 Hr	20 Hr	24 Hr
01	(b) (6)	BLQ	BLQ	82.5	182	171	134	80.3	53.4	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
		BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
		BLQ	BLQ	BLQ	40.3	BLQ	40.3	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
		BLQ	BLQ	60.4	87.5	81.4	69.0	44.7	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
		BLQ	41.7	55.3	46.5	43.4	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
		BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
		BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
		BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
		BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
		BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	STD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Median	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Min	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	Max	BLQ	41.7	82.5	182.0	171.0	134.0	80.3	53.4	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ

Source: Study Report of CPN-102, Table 14.2.1

The mean concentration versus time curves of lotilaner after single and multiple doses in healthy subjects are shown in Figure 4.1-1.

Figure 4.1-1 Individual Plasma Clobetasol Propionate Concentrations (PK Population)



Reference ID: 5307156

Source: Study Report of CPN-102, Figure 11.1
TRMT1: one dose (1 drop) APP13007 0.05%; TRMT2: two doses (1 drop each) APP13007 0.05% administered 12 hours apart with concentration data following the second dose.

APP13007 0.05% was well tolerated following ocular instillation in Study CPN-201. There were two treatment emergent adverse events (TEAEs) reported by two subjects (heart palpitation and menstrual cramps) of mild to moderate severity that were not considered to be related to the study drug. No serious AE, severe AEs or deaths were reported. No AEs resulted in discontinuation of the study drug or withdrawal of a subject from the study. For details in clobetasol propionate safety profile, we defer to the clinical review.

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 CPN-102 entitled "Determination of Clobetasol Propionate in Human Plasma Using High Performance Liquid Chromatography with Tandem mass Spectrometry (LC-MS/MS)" (report number AP19-055-01). Based on this report, this method for analysis of clobetasol propionate in human plasma has been adequately validated.

Clobetasol propionate concentrations in K_2EDTA human plasma were determined using an LC-MS/MS assay with a validated quantitation range between 0.04 and 20.0 ng/mL. 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

Item	Results
Bioanalytical method validation report location	\\CDSESUB1\evsprod\NDA218158\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\ap19-055-add1-add2\ap19-055-add1-add2.pdf
Study name	Validation of a LC-MS/MS method for the quantitation of Clobetasol Propionate in Human Plasma
Analyte	Clobetasol 17-Propionate
Internal Standard (IS)	Clobetasol 17-Propionate-d_5
Biological Matrix (Anticoagulant)	Human plasma (K_2EDTA)
Selectivity	Interference <20% of mean analyte response and <5% of mean internal standard response in the LLOQ for 7 lots of blank human plasma, 3 lots of lipemic human plasma and 3 lots of hemolyzed human plasma.
OTC and OC Specificity and Quantitative Interference Check	Lack of significant interference (<20% of mean analyte response and <5% of mean internal standard response in the LLOQ) and lack of impact on the quantitation of Clobetasol Propionate from OTC and OC medications was demonstrated.
Calibration Range	0.04 to 20 ng/mL

Intra-Run Accuracy (%Nominal)	LLOQ (0.04 ng/mL): 101.4% LQC (0.12 ng/mL): 98.6% MQC1 (4.8 ng/mL): 97.7% MQC2 (10.0 ng/mL): 98.4% HQC (16.0 ng/mL): 100.3% ULOQ (20.0 ng/mL): 97.6%
Intra-Run Precision (%CV)	LLOQ (0.04 ng/mL) : 3.0% LQC (0.12 ng/mL) : 2.0% MQC1 (4.8 ng/mL): 1.0% MQC2 (10.0 ng/mL): 0.5% HQC (16.0 ng/mL): 0.7% ULOQ (20.0 ng/mL): 2.0%
Inter-Run Accuracy (%Nominal)	LLOQ (0.04 ng/mL): 99.3% LQC (0.12 ng/mL): 101.7% MQC1 (4.8 ng/mL): 101.8% MQC2 (10.0 ng/mL): 102.9% HQC (16.0 ng/mL): 102.0% ULOQ (20.0 ng/mL): 97.6%
Inter-Run Precision (%CV)	LLOQ (0.04 ng/mL): 5.4% LQC (0.12 ng/mL): 3.6% MQC1 (4.8 ng/mL): 3.4% MQC2 (10.0 ng/mL): 3.6% HQC (16.0 ng/mL): 2.7% ULOQ (20.0 ng/mL): 2.0%
Regression Model	Linear, $1/x^2$
Sensitivity	Sensitivity at the LLOQ (0.04 ng/mL) was >5 times that of the peak area ratio of the ZS samples.
Carryover	No significant carryover observed.
Recovery	Clobetasol Propionate 78.9% Clobetasol Propionate-d_5 84.7%
Matrix Factor	No significant difference in the IS normalized matrix factor for 6 different lots of human plasma, as well as the 3 lots of hemolyzed plasma and 3 lots of lipemic plasma samples at the LQC and HQC levels.
Matrix Effect	No significant matrix effect for 6 different lots of human plasma, as well as the 1 lot of hemolyzed plasma and 1 lot of lipemic plasma samples at the LQC and HQC levels.
Dilution Integrity	40.0 ng/mL with 10-fold dilution
Whole Blood Stability	2 hours at 5˚C ± 3˚C
Bench-Top Stability	24 hours at room temperature and 5˚C ± 3˚C (LQC, HQC and DQC)
Freeze-Thaw Stability	3 Freeze-Thaw cycles, frozen at -70˚C ± 10˚C, thawed at room temperature (LQC, HQC and DQC)
Long Term Stability in Matrix	227 days at -70˚C ± 10˚C (LQC, HQC and DQC)
Autosampler Stability	71 hours at 5˚C ± 3˚C

Processed Sample Stability	2 hours at room temperature followed by 70 hours at 5˚C ± 3˚C
Reinjection Reproducibility	55 hours at 5˚C ± 3˚C
Long Batch	An accuracy and precision run containing 188 samples in total was assessed and met acceptance criteria.
Solution Stability	Stock solution: 6 hours at room temperature and 38 days at -20˚C ± 5˚C Working solution: 6 hours at room temperature and 13 days at -20˚C ± 5˚C IS stock solution: 6 hours at room temperature and 16 days at -20˚C ± 5˚C IS intermediate solution: 6 hours at room temperature and 15 days at -20˚C ± 5˚C

Bioanalytical Report Summary

The Applicant submitted one bioanalytical report detailing the performance of the PK sample analysis method used in a trial entitled "An Open-Label, Sequential Dosing Study to Evaluate Systemic Drug Exposure following Ocular Instillation of 0.05% APP13007 in Healthy Subjects" (protocol number CPN-102). Based on this report, the PK assay performed with acceptable precision and accuracy in Study CPN-102.

In total, 352 samples were analyzed in PK study CPN-102. Samples were received on August 04, 2020. Samples were analyzed between August 04, 2020 and August 07, 2020. All samples were kept at -70°C ± 10°C. The total duration of sample storage was 45 days and therefore falls within the validated time frame for long-term stability (227 days) at -70°C ± 10°C.

Eight-point calibration curves (linear regression; $1/X^2$ weighting) with concentrations ranging from 0.04 to 20.0 ng/mL were run in quadruplicate 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 (%Nominal) were ≤ 6.3% and ≤ 102.8%, respectively.

QCs at concentrations of 0.12 (LQC), 4.8 (LMQC), 10.0 (MQC) 16.0 ng/mL (HQC) were run in duplicate with each sample analysis run. Across all QC values in all sample runs, inter-assay QC precision (%CV) and inter-assay QC Accuracy (%Nominal) were 1.9 - 4.8% and 98.8 - 100.8%, respectively.

Incurred sample reanalysis (ISR) of 46 samples (approximately 13% of the total samples analyzed) yielded a passing rate of 100%. The Applicant's ISR evaluation is acceptable.

No carryover was observed for all runs.

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/s/

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