CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

217469Orig1s000

MULTI-DISCIPLINE REVIEW

Summary Review
Clinical Review
Non-Clinical Review
Statistical Review
Clinical Pharmacology Review

MEDICAL OFFICER, CROSS DISCIPLINE TEAM LEADER, and DIVISION DIRECTOR REVIEW OF NDA 217469

Application Type	NDA
Application Number(s)	NDA 217469 IND 128163
Priority or Standard	Standard
Submit Date(s)	August 8, 2022
Received Date(s)	August 8, 2022
PDUFA Goal Date	June 8, 2023
Division/Office	Division of Ophthalmology/Office of Specialty Medicine
Reviewer Name(s)	Rhea A. Lloyd, MD, William M. Boyd, MD, Wiley A. Chambers, MD
Review Completion Date	See DARRTS stamp date
Established/Proper Name	cyclosporine ophthalmic solution, 0.1%
Trade Name	Veyve
Applicant	Novaliq GmbH
Dosage Form(s)	Ophthalmic solution
Proposed Dosing Regimen(s)	Instill one drop of Veyve in each eye twice daily (at least 12 hours apart).
Applicant Proposed Indication(s)/Population(s)	Treatment of the signs and symptoms of dry eye disease.
Regulatory Action	Approval

Material Reviewed/Consulted OND Action Package, including:	Names of discipline reviewers
Regulatory Project Manager	Crystal Bland
Medical Officer Review	Rhea Lloyd
Statistical Review	Nam Hee Choi
Pharmacology Toxicology Review	Erin Ruhland
OPQ Review	Chunchun Zhang
Microbiology Review	Yarery Smith
Clinical Pharmacology Review	Amer Al-Khouja
OPDP	Carrie Newcomer
OSI	Roy Blay
CDTL Review	Rhea Lloyd
OSE/DMEPA	Lola Fashina
Labeling	Derek Alberding

OND=Office of New Drugs
OPQ=Office of Pharmaceutical Quality
OPDP=Office of Prescription Drug Promotion
DMPP=Division of Medical Policy Programs
OSI=Office of Scientific Investigations
CDTL=Cross-Discipline Team Leader
OSE= Office of Surveillance and Epidemiology
DEPI= Division of Epidemiology
DMEPA=Division of Medication Error Prevention and Analysis

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Glossary

AC	advisory committee
AE	adverse event
AR	adverse reaction
BLA	biologics license application
BPCA	Best Pharmaceuticals for Children Act
BRF	Benefit Risk Framework
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CDTL	Cross-Discipline Team Leader
CFR	Code of Federal Regulations
CMC	chemistry, manufacturing, and controls
COSTART	Coding Symbols for Thesaurus of Adverse Reaction Terms
CRF	case report form
CRO	contract research organization
CRT	clinical review template
CSR	clinical study report
CSS	Controlled Substance Staff
DMC	data monitoring committee
ECG	electrocardiogram
eCTD	electronic common technical document
ETASU	elements to assure safe use
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDASIA	Food and Drug Administration Safety and Innovation Act
GCP	good clinical practice
GRMP	good review management practice
ICH	International Council for Harmonization
IND	Investigational New Drug Application
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent to treat
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Event
NDA	new drug application
NME	new molecular entity
OCS	Office of Computational Science
OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology

OSI	Office of Scientific Investigation
PBRER	Periodic Benefit-Risk Evaluation Report
PD	pharmacodynamics
PI	prescribing information or package insert
PK	pharmacokinetics
PMC	post-marketing commitment
PMR	post-marketing requirement
PP	per protocol
PPI	patient package insert
PREA	Pediatric Research Equity Act
PRO	patient reported outcome
PSUR	Periodic Safety Update report
REMS	risk evaluation and mitigation strategy
SAE	serious adverse event
SAP	statistical analysis plan
SGE	special government employee
SOC	standard of care
TEAE	treatment emergent adverse event

1. Executive Summary

1.1. Product Introduction

Cyclosporine is a calcineurin inhibitor with relatively selective immunomodulatory properties. It is believed to act by suppressing the ocular surface immune reaction thereby increasing tear production. CyclASol is a clear solution of 0.1% (w/v) cyclosporine in a water-free excipient perfluorobutylpentane (abbreviated as F4H5), a semifluorinated alkane, and (b) (4) ethanol, (b) (4) (b) (4)

The applicant has submitted a 505(b)(2) application for the treatment of the signs and symptoms of dry eye disease. For the nonclinical safety, the applicant relies in part of the FDA's findings of efficacy and safety for the listed drug Sandimmune (NDA 50573). Additional nonclinical studies with CyclASol were conducted by Novaliq and demonstrated negligible systemic exposure.

This application seeks approval for treatment of the signs and symptoms of dry eye disease. Studies CYS-002, CYS-003 and CYS-004 were submitted in support of the application. The safety of CyclASol 0.1% was established by the submitted studies. The most common adverse reaction reported in (>5%) patients is instillation site reactions.

Regarding efficacy, studies CYS-003 and CYS-004 were successful in meeting their primary sign endpoint, the change from baseline in total corneal fluorescein staining at Day 29; however, a change in total corneal staining is not considered to be clinically significant unless either all staining is resolved (i.e., total clearing) or the change is associated with a change in symptoms. Neither of these studies was successful in meeting their primary symptom endpoint, the change from baseline in ocular surface disease index at Day 29 for CYS-003 and the change from baseline in eye dryness (VAS) at Day 29 for CYS-004. The applicant submitted a post hoc analysis of a different endpoint that has been accepted by the Agency for multiple other topical ophthalmic cyclosporine applications (the proportion of patients who achieved ≥ 10mm increase in Schirmer Tear Test at 5 min). This endpoint demonstrated a statistically significant difference relative to vehicle at Day 29 in both CYS-002 and CYS-004. The endpoint was also successful at Day 85 in CYS-003. The Division considers this endpoint to be the recommended endpoint for demonstrating an increase in tear production of cyclosporin containing topical ophthalmic products for the treatment of dry eyes.

1.2. Conclusions on the Substantial Evidence of Effectiveness

Studies CYS-002 and CYS-004 in NDA 217469 for CyclASol established the efficacy of Veyve (cyclosporine ophthalmic solution) 0.1 % for the treatment of dry eye disease. The regulatory action for this NDA will be APPROVAL.

NDA 217469 Benefit-Risk Integrated Assessment

The results of the clinical studies, CYS-002 and CYS-004, submitted in this NDA demonstrated that Veyve (cyclosporine ophthalmic solution) 0.1 % was statistically superior compared to vehicle in increasing tear production as measured by a 10mm increase in Schirmer tear test at Day 29.

The safety of Veyve (cyclosporine ophthalmic solution) 0.1 %was assessed in over 585 subjects dosed twice a day for 84 days. The safety profile was consistent with the known safety profile of cyclosporine ophthalmic products for topical administration. The most common adverse events experienced during the clinical studies was instillation site reactions 8%.

Benefit-Risk Dimensions

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Dry eye disease (DED) is a multifactorial, age-related, chronic disease of the ocular surface. • Chronic DED can cause severe pain, visual impairment, decreased tear production, tear film hyperosmolarity and instability. • Patients with corneal staining are more susceptible than others to eye infections and damage to the surface of the eye (cornea).	Cyclosporine is a calcineurin inhibitor believed to act by suppressing the ocular surface immune reaction thereby increasing tear production.
Current Treatment Options	Management of Dry Eye Disease may include: • Restasis (cyclosporine ophthalmic emulsion) 0.05% • Xiidra (lifitegrast ophthalmic solution) 5% • Cequa (cyclosporine ophthalmic solution) 0.09% • (b) (4) (varenicline tartrate nasal spray) • Miebo (perfluorohexyloctane ophthalmic solution) • OTC monograph products	Vevye (cyclosporine ophthalmic solution) 0.1% would provide an alternative product for the treatment of DED by increasing tear production.
Benefit	• Vevye (cyclosporine ophthalmic solution) 0.1% demonstrated a clinically significant improvement in tear product.	The amount of change in tearing has been associated with a change in clinical symptoms.
Risk and Risk Management	• The most common adverse events experienced by patients taking Vevye (cyclosporine ophthalmic solution) 0.1% was instillation site reactions (8%).	The safety profile demonstrated in the submitted studies was consistent with the known safety profile of other cyclosporine topical ophthalmic products.

1.3. Patient Experience Data

Clinical Trials CYS-002, CYS-003 (ESSENCE-1) and CYS-004 (ESSENCE-2):
Patient Experience Data Relevant to this Application (check all that apply)

☒	The patient experience data that was submitted as part of the application include:			Section where discussed, if applicable
	☒	Clinical outcome assessment (COA) data		See Section 6 of this review, Study Endpoints
		☒	Patient reported outcome (PRO)	
		☐	Observer reported outcome (ObsRO)	
		☒	Clinician reported outcome (ClinRO)	
		☐	Performance outcome (PerfO)	
	☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)		
	☐	Patient-focused drug development or other stakeholder meeting summary reports		
	☐	Observational survey studies designed to capture patient experience data		
	☐	Natural history studies		
	☐	Patient preference studies (e.g., submitted studies or scientific publications)		
	☐	Other: (Please specify)		
☐	Patient experience data that were not submitted in the application, but were considered in this review:			
		☐	Input informed from participation in meetings with patient stakeholders	
		☐	Patient-focused drug development or other stakeholder meeting summary reports	
		☐	Observational survey studies designed to capture patient experience data	
		☐	Other: (Please specify)	
☐	Patient experience data was not submitted as part of this application.			

2. Therapeutic Context

2.1. Analysis of Condition

Dry eye disease (DED) is a multifactorial, age-related, chronic disease of the ocular surface resulting in pain, and visual impairment. Dry eye disease affects the ability to carry out activities of daily living including reading, driving, and the ability to tolerate contact lenses. Patients with corneal staining are more susceptible than others to eye infections and damage to the surface of the eye (cornea). Dry eye disease is characterized by a reduction in tear volume, rapid breakup of the tear film, or an increase in the evaporative properties of the tear film layer. DED has a higher prevalence among women than men and increasing prevalence with age.

2.2. Analysis of Current Treatment Options

Summary of Treatment Armamentarium Relevant to Proposed Indication

NDA	Product (s) Name	Relevant Indication	Frequency of Administration
50790	Restasis (cyclosporine (ophthalmic emulsion) 0.05%	To increase tear production in patients whose tear production is presumed to be suppressed due to ocular inflammation associated with keratoconjunctivitis sicca	One drop twice a day in each eye approximately 12 hours apart
208073	Xiidra (lifitegrast ophthalmic solution) 5%	Treatment of the signs and symptoms of dry eye disease (DED)	One drop twice a day in each eye approximately 12 hours apart
210913	Cequa (cyclosporine ophthalmic solution) 0.09%	To increase tear production in patients with keratoconjunctivitis sicca (dry eye)	One drop twice a day in each eye approximately 12 hours apart
213978	Tyrvaya (varenicline solution) nasal spray	Treatment of dry eye disease	Spray in each nostril twice daily (approximately 12 hours apart).
216675	Miebo (perfluorohexyloctane ophthalmic solution)	Treatment of dry eye disease	One drop four times per day in each eye.
	Multiple OTC demulcents		

3. Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

This product has not been previously marketed.

3.2. Summary of Presubmission/Submission Regulatory Activity

Vevye (cyclosporine ophthalmic solution) 0.1% was developed under IND 128163 submitted to the Agency in November 2015.

Regulatory interactions with the Food and Drug Administration

FDA meeting type	Date	Discussion item
PIND	2015-06-16	General discussion on clinical development options under 505(b)(2) and possible endpoints. Agreement on Phase 2 study design.
EOP2A	2017-05-17	Agreement on dose selection for the program. Agreement on CYS-003 study design including primary endpoints and statistical methods.
Type C	2019-01-22	Discussion of development options based on CYS-003 results related to sign and symptom outcome.
EOP2	2019-07-15	Agreement on CYS-004 study design including primary endpoints and statistical methods. Furthermore, CYS-003 and CYS-004 together were considered sufficient clinical evidence for filing an NDA.
Type C	2020-04-24	Discussion on possibility to include (b) (4) into the CYS-004 study. Based on the feedback received Novaliq did not pursue this option further.
Pre-NDA Meeting	2022-02-28	Confirmation that CYS-003 and CYS-004 are considered sufficient clinical evidence for filing an NDA. Both studies met their primary sign endpoint demonstrating statistical superiority in change from baselines at Day 29. Neither study met its prespecified primary symptom endpoint. The percentage of subjects achieving a 10-mm increase or more in Schirmer's tear test, a single endpoint to demonstrate efficacy in the indication DED, was statistically significantly higher in the CyclASol group in both studies (secondary analyses in CYS-004 and post-hoc in CYS- 003)

3.3. Foreign Regulatory Actions and Marketing History

Vevye (cyclosporine ophthalmic solution) 0.1% is not marketed in any country.

4. Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigations (OSI)

From the OSI Clinical Inspection Summary finalized on March 21, 2023:

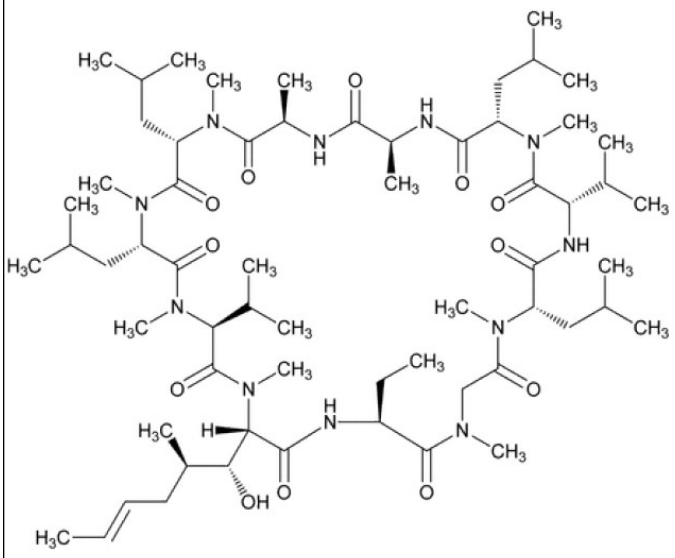
> The clinical sites of Drs. Pattar and Downing were inspected in support of this NDA. Based on the results of these inspections, Protocols CYS-003 and CYS-004 appear to have been conducted adequately and the data generated by these sites appear acceptable in support of the respective indication. Both sites were classified as No Action Indicated.

4.2. Product Quality

OPQ completed their integrated review of the application on May 12, 2023.

DRUG SUBSTANCE

Technical summary of drug substance Cyclosporine

INN	Ciclosporin
Compendial name	Cyclosporine (USP) Ciclosporin (Ph Eur, JP)
CAS registry number	59865-13-3
Chemical name	• cyclo[[(E)-(2S,3R,4R)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L- 2-aminobutyryl-N-methylglycyl-N-methyl-L-leucyl-L-valyl-N-methyl-L- leucyl-L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl- L-valyl].
Structure	
Molecular formula	$C_{62}H_{111}N_{11}O_{12}$
Molecular weight	1202.61 g/mol
Appearance	White or almost white powder
Solubility	Soluble in acetone, ethanol, methanol, ether, chloroform and dichloromethane; slightly soluble in saturated hydrocarbons; practically insoluble in water.
Therapeutic category	Immunosuppressant
Storage condition	Store at controlled room temperature

DRUG SUSBSTANCE SPECIFICATION

Cyclosporine is specified according to the current USP.

TEST	SPECIFICATION
Identification: • Infrared Spectroscopy • HPLC	Sample spectrum complies with spectrum of reference substance comply with the standard
Loss on Drying	NMT 2.0%
Related substances HPLC: Any individual impurity Total impurities	 NMT 0.7% NMT 1.5 %
Assay, on dried basis (HPLC)	between 97.0 % and 101.5 %

DRUG PRODUCT

Qualitative and quantitative composition of cyclosporine ophthalmic solution

		Quantity per Unit [mg/ 2mL]	Quantity per mL [mg]
Active ingredient	Function		
Cyclosporine (USP)	API	2.00	1.00
Excipients			
Perfluorobutylpentane (Inhouse)	(b) (4)		
Ethanol, (b) (4) (USP)			

Density (20°C) of solution: 1.29 g/cm^3

Release and shelf-life specifications for Cyclosporine ophthalmic solution

Test	Analytical Procedure	Acceptance Criteria for Release	Acceptance Criteria for Shelf-life
Appearance	Visual inspection Ph. Eur. 2.2.1, 2.2.2	Clear, colourless solution	Clear, colourless solution
Visible Particles	Visual inspection USP <790> (Inspection procedure), Ph. Eur. 2.9.20	Practically free from particles	Practically free from particles
Particulate matter	USP <788> Stage 1 (light obscuration method)	NMT (b) (4) particles per container ≥ (b) (4) µm NMT (b) (4) particles per container ≥ (b) (4) µm	NMT (b) (4) particles per container ≥ (b) (4) µm NMT (b) (4) particles per container ≥ (b) (4) µm

Filling Volume	Weighing	NLT (b) (4) mL	n.a.
Weight loss	Weighing	n.a.	NMT (b) (4)
Container Integrity	Visual inspection	No physical distortion or leaking	No physical distortion or leaking
Cyclosporine	HPLC in house (AM0830)	Retention time corresponds with reference substance (retention time shift is within ± 5% of reference standard) DAD spectrum corresponds with reference substance	n.a.
Cyclosporine	HPLC in house (AM0830)	(b) (4) mg/mL ((b) (4) of the labelled claim)	(b) (4) mg/mL ((b) (4) of the labelled claim)
Each specified Unknown impurity RRT (b) (4), RRT (b) (4), RRT (b) (4), RRT (b) (4), RRT (b) (4), RRT (b) (4), RRT (b) (4) Each unspecified unknown Impurity	HPLC in house (AM0830)	NMT (b) (4) % NMT (b) (4) %	NMT (b) (4) % NMT (b) (4) %
Total Impurities	HPLC in house (AM0830)	NMT (b) (4)	NMT (b) (4)
Sterility	USP<71>, Ph. Eur. 2.6.1, JP 4.06	Sterile	Sterile
(b) (4)	HPLC in house (AM0830)	NMT (b) (4) µg/mL	NMT (b) (4) µg/mL
	HPLC in house (AM0956)	NMT (b) (4) µg/mL	NMT (b) (4) µg/mL
	HPLC in house (AM0830)	NMT (b) (4) µg/mL	NMT (b) (4) µg/mL

n.a. = not applicable; NMT = not more than; NLT = not less than. *: known Impurities carried over from the API.

Reviewer's Comments: *The particulate matter specification is unnecessary.*

Technical summary of Perfluorobutylpentane

CAS registry number	1190430-21-7
Chemical name (IUPAC)	1,1,1,2,2,3,3,4,4-Nonafluorononane, 1-(Perfluorobutyl)pentane, Perfluorobutylpentane (F4H5)
Chemical class	Perfluoroalkane
Structure	
Molecular formula	$C_9H_{11}F_9$
Molecular weight	290.17 g/mol
Appearance	Clear colorless liquid
Solubility	Practically insoluble in water (0.06 mg/L). Sparingly miscible with Dimethyl sulfoxide, Dimethylformamide, 1,2-Propandiol. Miscible with Ethanol, Methanol, 2-Propanol, Petroleum ether, Acetone, Chloroform, Methylene chloride, Cyclohexane and Diethyl ether, 1-Methoxy-2-propanol, Acetaldehyde, Tetrahydrofurane, Ethyl acetate, Paraffin (25-60 mPas)
Polymorphism	N/A
Isomers	Perfluorohexyloctane does not exhibit stereoisomerism, as it does not contain asymmetric carbon atoms.
Storage condition	Store at controlled room temperature

Overview of CyclASol ophthalmic solution primary packaging components

Component	Information	
Bottle (b) (4) (b) (4)	Size	5 mL
	Description	Round plastic dropper bottle, polypropylene (PP), (b) (4) (drawing see Figure 1)
	Manufacturer/Type	(b) (4)
	DMF No.	
	Colorant	
	Production	
	Manufacturer	
	DMF No.	
Dropper (b) (4) (b) (4)	Duct diameter	(b) (4) mm
	Description	Plastic dropper, (b) (4) polyethylene (b) (4), (b) (4) (drawing see Figure 2)
	Manufacturer/Type	(b) (4)
	DMF No.	
	Colorant	
	Production	
	Manufacturer	

	DMF No.	(b) (4)
	Description	Screw cap, (b) (4) polyethylene (b) (4), white (drawing see Figure 3)
Cap (b) (4) (b) (4)	Manufacturer/Type	(b) (4)
	DMF No.	
	Colorant:	
	Manufacturer Type	
	DMF No.	
	Production	
	Manufacturer	
	DMF No.	

MICROBIOLOGY

The applicant has provided adequate sterility assurance. The drug product is (b) (4) (b) (4). Sterility is tested for release of finished drug product.

CDRH

CDRH stated that no review was necessary.

Facilities Table

Facility name and address	FEI	Responsibilities and profile code(s)	Status
Curia Italy S.r.l. Via Volturno 41/43 , Rozzano (MI), not applicable, Italy, 20089	3002807919	•Manufacture of drug substance and packaging• (b) (4)	Approve - Based on Previous History
Alliance Medical Products, Inc. / DBA Siegfried Irvine 9342 Jeronimo Road , Irvine, CA, USA, 92618	2027189	•Manufacture of drug product and packaging• (b) (4) (b) (4)	Approve - Based on PAI
HWI Pharma Services GmbH Rheinzaberner Strasse 8 , Ruelzheim, not applicable, Germany, 76761	3011258873	(b) (4)	Approve - Based on Previous History
Labor LS SE & Co. KG Mangelsfeld 4, 5, 6 , Bad Bocklet, not applicable, Germany, 97708	3002807481		Approve - Based on Previous History

Note: The facility table does not include the identification of the manufacturing facility for the perfluorobutylpentane. In Module 3.2.A. Appendix/3.2.A.3 Excipients the applicant provides the perfluorobutylpentane document which includes the manufacturer information for (b) (4) (b) (4) The excipient is not a critical excipient per the Drug Product review.

OPQ RECOMMENDATIONS AND CONCLUSION ON APPROVABILITY

Satisfactory information and responses have been submitted to support the drug substance, drug product, manufacturing process and quality microbiology aspects.

The product is regulated as a drug and device combination product per the Genus decision. However multi-dose vials are considered low risk and CDRH confirmed that a CDRH consult is not necessary on 8/16/2022.

The compliance status of the drug product manufacturing facility, Alliance Medical Products, Inc. (FEI: 2027189, Irvine, CA) was determined acceptable based on the most recent inspection performed ending May 5, 2023. Therefore, OPMA issued an overall recommendation of "Approval" on May 8, 2023. Therefore, NDA 217469 is recommended Approval from Product Quality perspective.

4.3. Nonclinical Pharmacology/Toxicology

From the Pharmacology/Toxicology review completed on May 8, 2023:

- The Applicant's relied on NDA 050573 (Sandimmune; Novartis Pharmaceutical Corp) for nonclinical support of systemic safety of CsA including genotoxicity, developmental and reproductive toxicity, and carcinogenicity. A bridge was established based on the large dose margin of the Listed Drug administered by intravenous injection and the Applicant's formulation, a topical ophthalmic drop.

- The Applicant's formulation is a solution primarily composed of the excipient perfluorobutylpentane. The Applicant conducted systemic and ocular toxicology studies of perfluorobutylpentane to qualify its use in the clinical formulations. At clinically relevant doses, no adverse toxicity was associated with perfluorobutylpentane. Perfluorobutylpentane was also negative for genotoxicity across *in vitro* and *in vivo* test systems.

- The Sponsor conducted a 6-month ocular toxicity study of the clinical formulation in rabbits. No adverse toxicity was associated with the vehicle or test article at doses which exceed the proposed clinical dose by 4.5-fold.

  The NDA is approvable from a nonclinical pharmacology/toxicology perspective.

4.4. Clinical Pharmacology

From the Clinical Pharmacology review of this application dated, March 27, 2023: The Office of Clinical Pharmacology has reviewed the clinical pharmacology data submitted in support of NDA 217469 and found the application acceptable to support approval from a clinical pharmacology perspective. The key review issues with specific clinical pharmacology recommendations and comments are summarized in the Table below.

Summary of clinical pharmacology review issues and recommendations

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	Cyclosporine ophthalmic solution, 0.1% w/v is administered as an eye drop and is locally acting. As a result, systemic exposure does not impact the treatment effect. Clinical pharmacology information does not provide pivotal or supportive evidence of effectiveness.
General dosing instructions	One drop twice a day in each eye approximately 12 hours apart
Dosing in patient subgroups (intrinsic and extrinsic factors)	No dosage adjustment in any patient subgroups is needed
Bridge between the to-be- marketed and clinical trial Formulations	The to-be-marketed formulation was used in the pivotal clinical studies.

The PK of cyclosporine after ophthalmic administration has previously been evaluated for approved products (i.e., Cequa, Verkazia, Restasis). For all three products, blood concentrations of cyclosporine were either undetectable (i.e., below the lower limit of quantitation [LLOQ]) in all patients, or undetectable in a proportion of patients. Detectable concentrations were only marginally above the LLOQ.

The PK of cyclosporine after ophthalmic administration was assessed in CYS-001 and CYS-002. Study CYS-001, conducted in healthy subjects, used a 0.05% w/v drug product formulation consisting of 0.5 mg/mL cyclosporine in perfluorobutylpentane with (b) (4)% w/w ethanol. Two different formulations were assessed in dose-ranging study CYS-002, which was conducted in subjects with dry eye disease. The formulations included a 0.05% w/v formulation with (b) (4) w/w ethanol, and a 0.1% w/v formulation (1.0 mg/mL cyclosporine) with (b) (4) w/w ethanol. The latter 0.1% formulation is the to-be-marketed formulation.

In studies CYS-001 and CYS-002, cyclosporine blood concentrations were below the LLOQ (0.100 ng/mL) in all samples at all timepoints. Ophthalmic administration of cyclosporine using the proposed product yielded no measurable systemic exposure. This is consistent with observed PK from approved ophthalmic cyclosporine products.

4.5. Companion Diagnostic Issues

Not applicable.

4.6. Consumer Study Reviews

Not applicable.

5. Sources of Clinical Data and Review Strategy

5.1. Table of Clinical Studies

Study Identifier	Objective of Study	Study design and Type of Control	Test Products; Dosage Regimen; Route of Administration	Number (N) of Subjects (treated)	Duration of Treatment
CYS-001	Safety and tolerability, PK	Single-center, randomized, double-masked, vehicle-controlled, 2-period, 2-way cross-over	CyclASol 0.05% or vehicle Dosing regimen A: Day 1: single drop OD (one eye), Day 2 and 3: single drop OU BID Dosing regimen B: Days 9, 10, and 11: Two drops OU BID	N=18 (all treated with CyclASol)	11 days per period, wash-out of 5 days between A and B
CYS-002	Efficacy, safety and tolerability, PK	Multicenter, randomized, double-masked, vehicle-controlled, with open-label comparator arm (Restasis).	CyclASol 0.05%, CyclASol 0.1%, vehicle, or Restasis Single drop OU, BID	N=207 CyclASol 0.05%: N=51 CyclASol 0.1%: N=51 Vehicle: N=52 Restasis: N=53	112 days
CYS-003	Efficacy, safety and tolerability	Multicenter, randomized, double-masked, vehicle-controlled	CyclASol 0.1% or vehicle Single drop OU, BID	N=328 CyclASol 0.1%: N=162 Vehicle: N=166	84 days
CYS-004	Efficacy, safety and tolerability	Multicenter, randomized, double-masked, vehicle-controlled	CyclASol 0.1% or vehicle Single drop OU, BID	N=834 CyclASol 0.1%: N=423 Vehicle: N=411	29 days
CYS-005	Safety, tolerability and efficacy during long-term use	Multicenter, open-label, single arm extension study to CYS-004	CyclASol 0.1% Single drop OU, BID	N=200* (all CyclASol 0.1%)	12 months

BID=twice daily, DED=dry eye disease, N=number of subjects, OD=once daily, OU=both eyes, PK=pharmacokinetic.
Note: Except for studies CYS-001 and CYS-005, all studies included a 14-day run-in period with artificial tears (Systane Balance, Alcon).
* Two subjects were excluded from the CYS-005 safety population (SAF), they received their treatment kits on site but were lost to follow up directly after Visit 1 of the study and did not contribute any further safety data; it is unknown if they took the medication. Source: Module 5.2

5.2. Review Strategy

The Applicant's primary support for efficacy for Vevye (cyclosporine ophthalmic solution) 0.1 % for the treatment of dry eye disease is based on data from the following studies: CYS-002, ESSENCE-1 (CYS-003) AND ESSENCE-2 (CYS-004). Clinical data from each of these studies was reviewed to determine if safety and efficacy were demonstrated.

6. Review of Relevant Individual Trials Used to Support Efficacy

6.1 Study CYS-002

A Phase 2, multicenter, randomized, double-masked, placebo (vehicle)-controlled clinical study with an open label comparator arm to assess the efficacy, safety and tolerability of topical CyclASol® at two different concentrations for the treatment of moderate to severe Dry Eye Disease in patients not responding adequately to artificial tears

Study Design:

This was a phase 2, multicenter, randomized, double-masked (with an open label comparator) study designed to evaluate the efficacy and safety of 0.05% and 0.1% CyclASol Ophthalmic Solution compared to vehicle and Restasis in subjects with moderate to severe DED. Two hundred and seven (207) male and female subjects at least 18 years of age with a subject-reported history of dry eye in both eyes and meeting all other study eligibility criteria were randomized to receive BID treatment with CyclASol 0.05%, CyclASol 0.1%, vehicle, or Restasis in a 1:1:1:1 ratio.

The study consisted of two periods: a 14-day run-in period with SYSTANE BALANCE each eye (OU) twice a day (BID) and a 112-day treatment period. The scheduled study visits were: Visit 0 (Screening; Day -14 ± 2), Visit 1 (Baseline/ Randomization; Day 1), Visit 2 (Day 15 ± 1), Visit 3 (Day 29 ± 2), Visit 4 (Day 85 ± 3), and Visit 5 (Study Exit; Day 113 ± 3). Dry eye signs and symptoms and safety measures were assessed at baseline and at the four follow-up visits. The study was designed to explore a range of signs and symptoms of DED to gain an understanding of the possible treatment effects in comparison to vehicle and estimation of effect sizes to select the most appropriate dose and endpoint for phase 3 and will ensure that the endpoint is adequately powered. The study was conducted at 4 investigative sites in the US.

Objective:
To compare the safety, efficacy, and tolerability of CyclASol® Ophthalmic Solutions (0.05% and 0.1%) to vehicle and Restasis® for the treatment of the signs and symptoms of dry eye disease (DED).

List of Investigators

Site 50 Andover Eye Associates 138 Haverhill Street, Suite 104 Andover, MA 01810	Principal Investigator: Gail Torkildsen, MD Sub-Investigators: John Pietrantonio, OD, Kathleen Hoen, OD, Charles Leahy, OD, Jason Chin, OD	41
Site 51 MedRACS, LLC 300 Congress Street, Suite 203 Quincy, MA 02169	Principal Investigator: Helen Moreira, MD Sub-Investigators: Steven Nielsen, MD, Robert Jordan, OD	57
Site 52 Central Maine Eye Care 181 Russell Street Lewiston, ME 04240	Principal Investigator: John Lonsdale, MD	68
Site 53 Eye Research Foundation 520 Superior Avenue, Suite 235 Newport Beach, CA 92663	Principal Investigator: David Wirta, MD Sub-Investigators: Justin Aaker, MD	41

Study Population

Inclusion Criteria

To be considered for entry into the study, subjects must have:

- Been at least 18 years of age;
- Provided written informed consent;
- Had a subject reported history of DED in both eyes for at least 6 months prior to Visit 0;
- Used (within 30 days prior to Visit 0) over-the-counter and/or prescription eye drops for dry eye symptoms at Visit 0;
- Had a score of ≥ 40 on the dryness VAS at Visit 0 and Visit 1;
- Had a total corneal fluorescein staining score of ≥ 6 (e.g. sum of inferior, superior, central, nasal, and temporal) according to the NEI grading at Visits 0 and Visit 1;
- Had a total lissamine green conjunctival score (sum of temporal and nasal) of ≥ 2, based on the Oxford grading at Visits 0 and Visit 1;
    - Had a Schirmer's Test I score between ≥ 2 mm and ≤ 8 mm at Visits 0 and Visit 1;
    - Had at least one eye (the same eye) satisfy all criteria for f, g, and h above;
    - Been able and willing to follow instructions, including participation in all study assessments and visits.

Exclusion Criteria:

Subjects may not have:

- Had any clinically significant slit-lamp findings at Visit 0 that included trauma, Steven Johnson Syndrome, active blepharitis, meibomian gland dysfunction (MGD), lid margin inflammation that required therapeutic treatment, and/or in the opinion of the investigator may have interfered with study parameters;
- Had abnormal lid anatomy (incomplete eyelid closure, entropion, ectropion) or blinking function of the eye;

- Had DED secondary to scarring: irradiation, alkali burns, cicatricial pemphigoid, destruction of conjunctival goblet cells (as vitamin A deficiency);
- Had an ocular or periocular malignancy;
- Had a corneal epithelial defect > 1 mm^2;
- Had a history of herpetic keratitis;
- Had active ocular allergies or ocular allergies that were expected to be active during the study period;
- Been diagnosed with an ongoing ocular or systemic infection (bacterial, viral, or fungal), including fever and current treatment with antibiotics, at Visit 0 and Visit 1;
- Worn contact lenses within 3 months of Visit 0 or anticipated using contact lenses during the study;
- Used any eye drops within 2 hours of Visit 0 and Visit 1;
- Had a history of no response to previous topical Cyclosporine A and/or use of topical Cyclosporine A within 6 months prior to Visit 0;
- Had intra-ocular surgery or ocular laser surgery within the previous 6 months, or had any planned ocular and/or lid surgeries over the study period;
- Been a woman who was pregnant, nursing or planning a pregnancy;
- Been unwilling to submit a blood pregnancy test at Visit 0 and Visit 5 (or early termination visit) if of childbearing potential. Non-childbearing potential was defined as a woman who was permanently sterilized (e.g. has had a hysterectomy or bilateral tubal ligation or bilateral oophorectomy), or was post-menopausal (without menses for 12 consecutive months);
- Been a woman of childbearing potential who was not using an acceptable means of birth control; acceptable methods of contraception included: hormonal – oral, implantable, injectable, or transdermal contraceptives; mechanical – spermicide in conjunction with a barrier such as a diaphragm or condom; intrauterine device; or surgical sterilization of partner. For non-sexually active females, abstinence may have been regarded as an adequate method of birth control; however, if the subject became sexually active during the study, she must have agreed to use adequate birth control as defined above for the remainder of the study;
- Had an uncontrolled systemic disease;
- Had a known allergy and/or sensitivity to the study drug or its components (Cyclosporine A or SFA);
- Had active ocular or periocular rosacea and/or pterygium;
- Been currently enrolled in an investigational drug or device study or used an investigational drug or device within 60 days of Visit 0;
- Used any topical anti-glaucoma medication within 3 months prior to Visit 0;
- Used any topical ocular or facial steroids or serum tears within one month prior to Visit 0;
- Used systemic steroids including dermatological steroids with high potency or large treatment areas or immunomodulating agents on a non-stable regimen within 3 months prior to Visit 0 or expected to be unstable during the study period;

- Used any oral medications known to cause ocular drying (eg, antihistamines, antidepressants, etc.) on a non-stable regimen within 1 month prior to Visit 0 or expected to be unstable during the study;
- Had corrected visual acuity greater than or equal to logarithm of the minimum angle of resolution (logMAR) +0.7 as assessed by Early Treatment of Diabetic Retinopathy Study (ETDRS) scale in both eyes at Visit 0 and Visit 1;
- Had a condition or been in a situation (including language barrier) which the investigator felt may have put the subject at significant risk, may have confounded the study results, or may have interfered significantly with the subject's participation in the study;
- Had received a new punctum plug within 1 month prior to Visit 0 or expected to receive a punctum plug or removal of a punctum plug during the study.

Study Drugs Supplied

Test Article	Concentration (w/v)	Dose	Supplier	Lot Number
CyclASol	0.05%	BID	(b) (4)	150882
CyclASol	0.1%	BID		150883
Vehicle	NA	BID		150881
Restasis	0.05%	BID		92241A0, 92241B0
Abbreviations: BID = twice a day; NA = Not Applicable,				

Criteria for Evaluation:

Efficacy Measures:

Primary efficacy measures

- Corneal fluorescein staining total (NEI scale)
- Dryness severity visual analog scale (VAS)

Secondary efficacy measures

- Corneal fluorescein staining score sub-regions (NEI scale)
- Corneal fluorescein staining total and sub-regions (Ora Calibra™ scale)
- Conjunctival lissamine green staining (Oxford scale)
- Conjunctival lissamine green staining (Ora Calibra™ scale)
- Conjunctival redness (Ora Calibra™ scale)
- Unanesthetized Schirmer's Test I
- Tear film break up time (TFBUT)
- VAS for severity of burning/stinging, sticky feeling, foreign body sensation, itching, blurred vision, sensitivity to light, pain and frequency for dryness
- Ocular Discomfort Score (ODS; Ora Calibra™ scale)
- Ocular Discomfort & 4-Symptom Questionnaire (Ora Calibra™ scale)
- Ocular Surface Disease Index© (OSDI©)
- Contrast sensitivity (Ora Calibra™)
- Tear osmolarity
- Human Leukocyte Antigen – antigen D Related (HLA-DR) expression on the ocular surface

- Matrix metallopeptidase 9 (MMP9) levels (InflammaDry® test); and recorded in diary

Schedule of Events and Procedures

Procedure	Visit 0 Day -14 ± 2	Visit 1 Day 1	Visit 2 Day 15 ± 1	Visit 3 Day 29 ± 2	Visit 4 Day 85 ± 3	Visit 5 Day 113 ± 3
Informed Consent / HIPAA	X					
Medical / Medication History and Demographic	X					
Run-in artificial tears Collection		X				
Diary Collection		X	X	X	X	X
Medical / Medication History Update		X	X	X	X	X
Adverse Event Query	X	X	X	X	X	X
VAS	X	X	X	X	X	X
Ora Calibra™ Ocular Discomfort / Dry Eye Symptoms Questionaire	X	X	X	X	X	X
OSDI	X	X	X	X	X	X
Ora Calibra™ Contrast Sensitivity Test		X				X
Visual Acuity (ETDRS)	X	X	X	X	X	X
Review of Qualification Criteria	X	X				
Slit-lamp Biomicroscopy	X	X	X	X	X	X
Ora Calibra™ Conjunctival Redness	X	X	X	X	X	X
Tear Osmolarity	X	X				X
TFBUT	X	X	X	X	X	X
Fluorescein Staining- Ora Calibra™ /NEI Scales	X	X	X	X	X	X
Photographs of Staining		X				X
Lissamine Green Stain- Ora Calibra/Oxford Scale	X	X	X	X	X	X
InflammaDry	X	X				X
Schirmer's Test I (without anesthesia)	X	X	X	X	X	X
HLA-DR		X				X
Intraocular Pressure	X					X
Dilated Fundoscopy	X					X
Randomization		X				
Physical Examination[2]	X					
Blood Draw Safety Lab (Including Blood pregnancy test)	X					X
Run-in Instillation	X					
Run-in Dispensation	X					
Randomized Study Drug Instillation		X				X
Ora Calibra Drop Comfort Scale and Questionnaire		X				X
Blood Draw PK		X[1]				X[3]
Randomized Study Drug Dispensation		X	X	X	X	
Diary Dispensation	X	X	X	X	X	
Study Exit						X

[1]At one site, samples will be taken pre-dose and at 0.5, 1, 2, and 4 hours (±5 min for all time points) after instillation.
[2]Physical Examination includes HEENT, cardiovascular, respiratory, and dermatological examinations.
[3]Collected at one or two sites only.

Analysis Populations:
Full Analysis Set (FAS): included all subjects randomized and having received at least one dose of study drug; evaluated for all data available. The primary analysis was performed on the FAS. Subjects in the FAS were analyzed as treated.

Per Protocol (PP) population: included subjects in the FAS who did not have major protocol deviations that compromised subject safety or impacted the evaluability of the primary endpoint and who completed the study.

Safety population: included all subjects who received at least one dose of the study drug. The safety population was analyzed for all safety assessments.

Pharmacokinetic (PK) population: included all subjects with a PK predose and postdose blood draw. The PK population was used to summarize all Cyclosporine A and F4H5 blood concentrations.

Primary Efficacy Analysis:
The primary treatment comparisons in this study were between CyclASol (0.05% and 0.1%) versus vehicle. The total corneal fluorescein staining score (NEI scale) was calculated as the sum of the staining from the inferior, central, superior, nasal, and temporal regions, such that a larger number indicated worse dry eye signs.

Primary Efficacy Endpoints (e.g., change from baseline to Visit 5 [Day 113] in total corneal fluorescein staining [NEI scale] and dryness severity VAS) were analyzed separately using an analysis of covariance (ANCOVA) model with terms for baseline value, treatment (including all 4 treatment groups), and the interaction of treatment by baseline value. Treatment group least square means (LS means) differences from the model were presented with p-values (*P*) and two-sided 95% CIs.

Compliance with Good Clinical Practices
The study was conducted in accordance with the principles of ICH GCP, applicable regulatory requirements, and Sponsor/CRO Standard Operating Procedures. The study followed the recommendations of ICH GCP R2 with quality oversight provided by the sponsor to ensure human subject protection and reliability of trial results.

Financial Disclosure
The sponsor has certified that they have not entered into any financial arrangements with the investigators involved in this trial.

Subject Disposition

	CyclASol 0.05% (N=51)	CyclASol 0.1% (N=51)	Restasis (N=53)	Vehicle (N=52)
Full analysis set (FAS) (randomized subjects)	51	51	53	52
Safety population	51	51	53	52
Per Protocol (PP) population	47 (92%)	49 (96%)	49 (92%)	47 (90%)
Pharmacokinetic (PK) population	12 (23%)	17 (33%)	13 (24%)	13 (25%)
Total eyes	102	102	106	104
Qualifying eyes [1]	77 (75%)	80 (78%)	82 (77%)	81 (78%)
Subjects Completing the Study				
Yes	50 (98%)	50 (98%)	52 (98%)	48 (92%)
No	1 (2%)	1 (2%)	1 (2%)	4 (8%)
Subjects with Protocol Deviations [2]				
Protocol deviation	48 (94%)	40 (78%)	42 (79%)	47 (90%)
Major deviation	3 (6%)	1 (2%)	3 (6%)	1 (2%)
Minor deviation	47 (92%)	40 (78%)	41 (77%)	47 (90%)
Reason for Withdrawal [3]				
Adverse Events	1	0	1	2
Subject choice	0	0	0	2
Protocol Deviation	0	0	0	0
Administrative Reasons	0	0	0	0
Sponsor Termination of Study	0	0	0	0
Other	0	1	0	0

[1] A qualifying eye is one that met all applicable inclusion criteria.
[2] Classification of deviations as major/minor by the sponsor together with CRO occurred before database lock and unmasking.
[3] Percentages are based on the number of discontinuations.
Note: N in the headers represents the total number of subjects in each respective treatment group within the FAS population. All analysis populations were analyzed as treated.
Percentages for the FAS and PP populations are based on the number of randomized subjects as randomized. Percentages for the Safety population are based on the number of randomized subjects as treated. Percentages for the PK population are based on the number of randomized subjects who agreed to PK sampling.
Percentage for study completion, reason for discontinuation, protocol deviations are based on the total number of randomized subjects as randomized in each treatment group.
Source: CYS-002 CSR Table 5

Demographic Information by Treatment Groups – FAS Population

	CyclASol 0.05% (N=51)	CyclASol 0.1% (N=51)	Restasis (N=53)	Vehicle (N=52)
Age (years)				
Mean (SD)	64.3 (10.72)	61.1 (12.29)	62.8 (11.90)	61.3 (10.45)
Median	64.0	61.0	62.0	62.0
Min, Max	33, 85	30, 86	27, 94	26, 82
< 65 years	26 (51%)	32 (63%)	35 (66%)	31 (60%)
≥ 65 years	25 (49%)	19 (37%)	18 (34%)	21 (40%)
Gender				
Male	13 (25%)	15 (29%)	13 (25%)	13 (25%)
Female	38 (75%)	36 (71%)	40 (75%)	39 (75%)
Ethnicity				
Hispanic or Latino	1 (2%)	3 (6%)	2 (4%)	2 (4%)
Not Hispanic or Latino	50 (98%)	48 (94%)	51 (96%)	50 (96%)
Race				
American Indian or Alaskan Native	0	0	0	0
Asian	0	2 (4%)	2 (4%)	0
Black or African American	0	5 (10%)	5 (9%)	5 (10%)
Native Hawaiian or Other Pacific Islander	1 (2%)	0	0	0
White	50 (98%)	44 (86%)	46 (87%)	47 (90%)
Iris Color (Worse Eye)				
Blue	18 (35%)	20 (39%)	11 (21%)	17 (33%)
Brown	15 (29%)	22 (43%)	28 (53%)	23 (44%)
Hazel	11 (22%)	6 (12%)	11 (21%)	8 (15%)
Green	6 (12%)	3 (6%)	1 (2%)	4 (8%)
Gray	1 (2%)	0	2 (4%)	0

Note: N in the headers represents the total number of subjects in each respective treatment group within the FAS population. Source: Table 8 CSR-CYS-002

Baseline Characteristics – FAS Population

	CyclASol 0.05% (N=51)	CyclASol 0.1% (N=51)	Restasis (N=53)	Vehicle (N=52)
Total Corneal Fluorescein Staining Score (NEI scale)				
Mean (SD)	8.8 (2.1)	8.7 (1.9)	8.8 (1.9)	8.71 (2.1)
Median	9	9	9	9
Min, Max	6, 14	6, 13	6, 14	6, 14
Two-Sided 95% CI	(8.2, 9.4)	(8.2, 9.3)	(8.3, 9.3)	(8.1, 9.3)
Severity of Dryness (VAS)				
Mean (SD)	72.8 (19.5)	71.2 (16.2)	68.5 (18.8)	68.5 (21.4)
Median	80	71	70	70.5
Min, Max	20, 100	35, 100	30, 100	10, 100
Two-Sided 95% CI	(62.8, 78.3)	(66.6, 75.7)	(63.3, 73.7)	(62.6, 74.5)

Note: N in the headers represents the total number of subjects in each respective treatment group within the FAS population. The worst eye from each subject was selected based on baseline total corneal staining score. If both eyes had the same total corneal staining score, then the right eye was used. Corneal fluorescein staining (NEI scale) was assessed on a 0-3 scale for each region, with a score of 0 meaning no staining. Total corneal fluorescein staining score was the sum of scores

from inferior, superior, central, temporal, and nasal regions of the cornea. VAS symptoms were measured on both eyes simultaneously and were recorded as percentages, where 0% meant no dryness and 100% meant maximal dryness.
Baseline measures are defined as the last non-missing measure prior to the initiation of randomized study treatment.
Source: CSR CYS-002 Table 9

Primary Efficacy Endpoints

Change from Baseline in Total Corneal Fluorescein Staining (NEI scale) at Visit 5

	CyclASol 0.05%	CyclASol 0.1%	Vehicle
Visit 5 (Day 113)	N=50	N=50	N=48
Mean (SD)	6.46 (2.727)	6.64 (2.538)	7.10 (3.157)
Two-Sided 95% CI	(5.68, 7.24)	(5.92, 7.36)	(6.19, 8.02)
Mean difference [1]	-0.64	-0.46	
p-value (paired t-test vs. baseline)	0.2818	0.4235	
p-value (Wilcoxon rank-sum test)	0.5222	0.5541	
Change from Baseline			
Mean (SD)	-2.38 (2.156)	-2.10 (2.252)	-1.69 (2.619)
Two-Sided 95% CI	(-2.99, -1.77)	(-2.74, -1.46)	(-2.45, -0.93)
LS mean difference [1] (2-sided 95% CI)	-0.68 (-1.57, 0.21)	-0.42 (-1.32, 0.47)	
p-value, ANCOVA model	0.1334	0.3505	

Source: CYS-002 CSR Table 11

Reviewer's Comment: *The CyclASol 0.1% treatment group showed a numerically greater improvement in total corneal fluorescein staining compared to vehicle; however, the difference was not statistically significant.*

Change from Baseline in Dryness Severity (VAS) at Visit 5

	CyclASol 0.05%	CyclASol 0.01%	Vehicle
Visit 5 (Day 113)	N=50	N=50	N=48
Mean (SD)	52.66 (23.328)	54.76 (27.452)	51.15 (26.585)
Two-Sided 95% CI	(46.03, 59.29)	(46.96, 62.56)	(43.43, 58.87)
Mean difference [1]	1.51	3.61	
p-value (paired t-test vs. baseline)	0.7648	0.5098	
p-value (Wilcoxon rank-sum test)	0.8006	0.4464	
Change from Baseline			
Mean (SD)	-11.00 (19.362)	-9.30 (27.832)	-13.85 (25.301)
Two-Sided 95% CI	(-16.50, -5.50)	(-17.21, -1.39)	(-21.20, -6.51)
LS mean difference [1] (2-sided 95% CI)	2.4 (-7.2, 12.1)	4.3 (-5.4, 13.9)	
p-value, ANCOVA model	0.6166	0.3829	

Source: CYS-002 CSR Table 12

Reviewer's Comment: *The CyclASol groups showed numerical less reduction in the eye dryness severity score compared to the vehicle group.*

6.2 ESSENCE 1 - Study CYS-003

A Phase 2b/3, multicenter, randomized, double-masked, vehicle-controlled clinical study to assess the efficacy and safety of topical CyclASol for the treatment of signs and symptoms of Dry Eye Disease

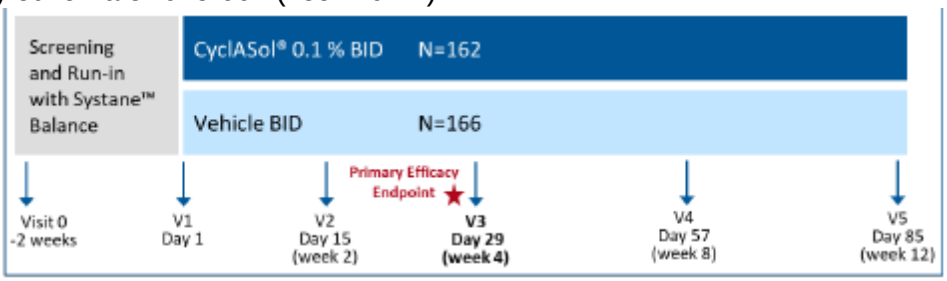
Study Design

This was a Phase 2b/3, multicenter, randomized, double-masked, vehicle-controlled clinical study to assess the efficacy and safety of topical CyclASol 0.1% for the treatment of DED. Subjects were eligible if their OSDI score was ≥20 and if one eye (the same eye) met the following main inclusion criteria at screening and time of randomization: tCFS ≥ 10 (NEI scale), total lissamine green conjunctival score of ≥ 2 (Oxford scale), and unanesthetized Schirmer's tear test score between ≥ 1 and ≤ 10 mm.

After a 14-day run-in period with lubricant eye drops (Systane Balance, Alcon) OU BID, subjects were randomized in a 1:1 ratio to CyclASol 0.1% or vehicle and were treated with study drug OU BID over an 84-day treatment period. A total of 316 subjects were planned to be enrolled (158 subjects per treatment arm). Scheduled visits included Visit 0 (Screening; Day -14), Visit 1 (Baseline/Randomization; Day 1), Visit 2 (Day 15), Visit 3 (Day 29), Visit 4 (Day 57), and Visit 5 (Study exit; Day 85). Additional symptoms were explored to inform future studies. The study was conducted at 9 investigative sites in the United States.

The two primary efficacy endpoints were change from baseline in tCFS (NEI scale) and change from baseline in OSDI, both assessed after 1 month of treatment

Study Schema of CYS-001 (ESSENCE-1)



List of Investigators
Subjects were enrolled at 9 sites in the United States.

Site 40 Total Eye Care, PA 6060 Primacy Parkway, Suite 200 Memphis, TN 38119	Principal Investigator: Eugene McLaurin, MD Sub-Investigators: David Evans, OD, Don Gayso, OD, Brandon Rushing, OD	55
Site 41 Eye Care Institute 1536 Story Avenue Louisville, KY 40206	Principal Investigator: Guruprasad Pattar, MD	30
Site 42 Eye Research Foundation 520 Superior Avenue, Suite 235 Newport Beach, CA 92663	Principal Investigator: David Wirta, MD Sub-Investigator: David Salvay, MD PhD	85
Site 43 East West Eye Institute 23441 Madison Street, Suite 120 Torrance, CA 90505	Principal Investigator: Alex Chunhsien Liu, MD Sub-Investigators: Michelle A. Sato, MD, Eric Ahn, MD	39
Site 44 Andover Eye Associates 138 Haverhill Street, Suite 104 Andover, MA 01810	Principal Investigator: Gail Torkildsen, MD Sub-Investigators: John Pietrantonio, OD Charles Leahy, OD, Jason Chin, OD	39
Site 45 Andover Eye Associates 675 Paramount Drive, Suite 305 Raynham, MA 02767	Principal Investigator: Kenneth Kenyon, MD Sub-Investigators: Robert Jordan, OD	8
Site 46 Nashville Vision Associates 4306 Harding Road, Suite 202 Nashville, TN 37205	Principal Investigator: Gary Jenkins, MD	19
Site 47 Virginia Eye Consultants 241 Corporate Boulevard Norfolk, VA 23502	Principal Investigator: John Sheppard, MD Sub-Investigators: Christopher Kruthoff, OD Jessica Schiffbauer, OD	10
Site 49 Midwest Cornea Assoc., LLC 10300 N Illinois Street, Suite 1020 Indianapolis, IN 46290	Principal Investigator: Blair Boehmer, MD	43

Diagnosis and Main Criteria for Inclusion:

- Adult subjects 40 to 55 years of age with objective and subjective evidence of presbyopia were eligible for enrollment.

Key inclusion criteria:

- Subjective complaints of poor near vision that impact activities of daily living, as defined by at least a moderate impact (score ≥ 3) on at least 1 question on the NEI VFQ-25 Questions 5 to 7 in the main questionnaire or Near Vision Subscale, Questions A3 to A5 in the Appendix of Optional Additional Questions, at the screening visit

- Best cycloplegic distance correction at screening in the range of spherical -4.00 D to +1.00 D inclusively and cylinder ≤ ±2.00 D with photopic, high-contrast binocular CDVA of 20/25 or better at the screening and baseline visits
- Photopic, high-contrast binocular CDVA of 20/32 or better by habitual monofocal correction (either spectacles or contact lenses), or willing to wear new monofocal correction spectacles to achieve photopic, high-contrast CDVA of 20/32 or better during the study
- Mesopic, high-contrast binocular DCNVA of 20/40 (J3) to 20/100 (J10) at the screening and baseline visits
- Photopic, high-contrast, near visual acuity correctable to 20/40 (J3+) or better in each eye at the screening visit
- Mesopic pupil diameter < 8.0 mm in both eyes at the screening visit

Key exclusion criteria:

- Severe dry eye disease (defined as total corneal staining ≥ grade 3 on the 5-point Oxford scale and an OSDI score of > 33) at the screening visit
- Corneal abnormalities (including keratoconus, corneal scar, Fuchs' endothelial dystrophy, guttata, or edema) in either eye that were likely to interfere with visual acuity
- Narrow iridocorneal angles (Shaffer grade ≤ 2 or lower on gonioscopy examination), history of angle closure glaucoma, or previous iridotomy
- History of iris trauma, Adie's tonic pupil, abnormal pupil shape in either eye, or anisocoria > 1 mm between pupils under mesopic conditions at the screening visit
- Lens opacity in either eye that was determined to cause significant disturbance of the central visual axis on screening biomicroscopy
- Diagnosis of any type of glaucoma or ocular hypertension
- Bifocal or multifocal spectacles or contact lenses for habitual correction. Subjects willing to wear study-provided monofocal correction (either spectacles or contact lenses) during the study could be enrolled.
- Had cataract surgery, phakic intraocular lens surgery, corneal inlay surgery, radial keratotomy, or any intraocular surgery. However, subjects with history of PRK or LASIK with CDVA meeting inclusion criteria were allowed to enroll.
- Presence of any ocular condition that, in the opinion of the investigator, could affect the safety of the subject or interpretation of efficacy parameters (e.g., uveitis, retinal detachment)
- Concurrent use of temporary or permanent punctal plugs or history of punctal cautery in one or both eyes

Test and Reference Therapies, Dose and Mode of Administration, Lot Number
Study Drugs Supplied

Test Article	Concentration (w/v)	Dose	Supplier	Lot Number
CyclASol	0.1%	BID	Alliance Medical Products, Inc.	1732-017
Vehicle	NA	BID	Alliance Medical Products, Inc.	1732-016 1737-032
Abbreviations: BID = twice a day; NA = Not Applicable,				

Rescue Medication
None. Disallowed medications and treatments were listed in the Exclusion Criteria. All medications and treatments that were not allowed prior to the study were not allowed during the study. In particular, no other dry eye treatment was allowed during the course of the study, such as artificial tears (other than run-in medication), gels, or ointments.

Criteria for Evaluation

Efficacy:
Primary efficacy
- Change from baseline (CFB) in total corneal fluorescein staining score (NEI scale) at Day 29
- Change from baseline in total OSDI score at Day 29

Key secondary efficacy measures
- Total corneal fluorescein staining score (NEI scale) and change from baseline to each measured post-baseline visit (other than Day 29).
- Total OSDI score and change from baseline to each measured post-baseline visit (other than Day 29).
- Lead/Worst symptom assessment.
- Reading impairment score and change from baseline to each measured post-baseline visit.
- Unanesthetized Schirmer's tear test responders and change from baseline to each measured post-baseline visit.
- Central and inferior corneal staining score (NEI scale) and changes from baseline to each measured post-baseline visit.

Study CYS-003 - Sequence of Events and Procedures

Procedure	Visit 0 Day -14 ±2	Visit 1 Day 1	Visit 2 Day 15 ±1	Visit 3 Day 29 ±2	Visit 4 Day 57 ±2	Visit 5 Day 85 ±2
Informed consent / HIPAA	X					
Medical/Medication history and demographics	X					X
Urine Pregnancy Test (as needed)[1]	X					X
Medical/Medication history update		X	X	X	X	X
Adverse event query	X	X	X	X	X	X
OSDI	X	X	X	X	X	X
Lead/Worst symptom selection and assessment		X	X	X	X	X
Reading impairment score	X	X	X	X	X	X
VAS	X	X	X	X	X	X
Reading assessments		X		X		X
Visual acuity (ETDRS)	X	X	X	X	X	X
Review of qualification criteria	X	X				
Slit-lamp biomicroscopy	X	X	X	X	X	X
TFBUT	X	X		X		X
Fluorescein staining - NEI Scale	X	X	X	X	X	X
Lissamine green staining - Oxford Scale	X	X		X		X
InflammaDry		X		X		X
Schirmer's tear test I (without anesthesia)	X	X	X	X	X	X
Intraocular pressure	X			X		X
Dilated fundoscopy	X					X
Randomization		X				
In-office instillation of run-in	X					
Run-in & diary dispensation	X					
Run-in collection		X				
In-office instillation of randomized study drug		X				
Drop Comfort Scale and Questionnaire		X				
Randomized study drug dispensation		X	X[2]	X	X	X
Randomized study drug collection			X	X	X	X
Diary review/ collection		X	X	X	X	X
Study exit						X

Abbreviations: ETDRS = Early Treatment of Diabetic Retinopathy Study; HIPAA = Health Insurance Portability and Accountability Act; NEI = National Eye Institute; OSDI = Ocular Surface Disease Index, TFBUT = tear film break-up time; VAS = visual analog scale.

[1] For women of childbearing potential.

[2] Re-dispensation of the bottle dispensed at Visit 1.

Statistical Analysis Plan

Analysis Populations

- The Full Analysis Set (FAS) – Same as Study CYS-002
- The Per Protocol Set (PPS) – Same as Study CYS-002
- The Safety Set (SAF) – Same as Study CYS-002

All statistical tests were two-sided with a significance level of 0.05 ($\alpha = 0.05$). Confidence intervals (CIs) for differences between the CyclASol and vehicle groups were two-sided at 95% confidence. For statistical analyses that used site as a fixed effect, any site that enrolled fewer than 10 subjects was pooled with the site with the next smallest enrollment.

Baseline measures were defined as the last non-missing measure prior to administration of study drug. In most cases, this was data from Visit 1. Change from baseline was calculated as follow-up visit minus baseline value.

Safety endpoints were analyzed for both eyes. For efficacy endpoints that were measured separately in each eye, the unit of analysis was the "study eye."

Eyes were eligible for analysis if they met all inclusion criteria. In the case that both eyes were eligible for analysis, the "study eye" was the eye with the higher mean corneal fluorescein staining score (NEI scale) at Visit 1. If the mean corneal fluorescein staining score (NEI Scale) was the same OU, then the right eye (OD) was designated as the "study eye."

Missing or Inconclusive Data Handling

The primary analysis used the FAS with available data per subject. Additional robustness analyses included repeating the primary analysis on the PPS, the FAS imputing missing data using Last Observation Carried Forward (LOCF), the FAS imputing missing data using Markov Chain Monte Carlo (MCMC) multiple imputation methodology under different assumptions of missingness (at random and not at random), and an analysis of trimmed means (Permutt et al, 2017).

Primary Efficacy Analysis:

The primary comparisons in this study were between CyclASol and vehicle at Day 29. The primary efficacy analysis compared the mean changes from baseline in total corneal fluorescein staining score (NEI scale) and OSDI score, which were analyzed separately using an analysis of covariance (ANCOVA) model with terms for subject baseline value, clinical study site, treatment, and the interaction of treatment by baseline value (the interaction term was maintained in the model only if the P-value (P) for the term was < 0.10).

Least squares (LS) means for each treatment group and for the difference between treatment groups are presented from the model together with two-sided Ps and 95% confidence intervals (CIs).

Safety Analysis:

AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 20.1. Frequencies and percentages of subjects with treatment-emergent adverse events (TEAEs), serious TEAEs, and TEAEs causing premature discontinuation are provided by treatment group. An AE was treatment emergent if it 1) occurred after the first dose of randomized study treatment or 2) if it was present prior to the receipt of randomized study treatment but worsened in severity or increased in frequency after the first dose of randomized study treatment. Frequencies are given of subjects with TEAEs by system organ class (SOC) and preferred term (PT); by SOC, PT, and maximal severity; by SOC, PT, and strongest relationship; by SOC and PT for serious adverse events (SAEs); and by SOC, PT, and day of onset. Separate analyses were performed for ocular TEAEs and all non-ocular TEAEs.

Other safety endpoints, including VA, slit lamp biomicroscopy, IOP, and dilated fundoscopy, are summarized by treatment group and visit using descriptive statistics. Changes or shifts from baseline also are summarized where appropriate. For assessments performed by eye, study eye (worst eye) and fellow eye are summarized separately.

Adjustments for Multiplicity

Fixed sequence testing was employed to maintain the type I error rate for the primary endpoints. There were no adjustments for secondary endpoints; thus, all secondary endpoints were considered exploratory.

The primary analyses first tested the difference in the mean change from baseline corneal fluorescein staining (NEI scale) total score between treatments at Day 29. If the test of the difference was statistically significant at the two-sided $\alpha = 0.05$ level in favor of CyclASol, then the study would be considered a success; CyclASol would be declared to be superior to the vehicle in the mean change from baseline corneal fluorescein staining (NEI scale) total score at Day 29; and the difference in the mean change from baseline total OSDI score between treatments at Day 29 would be tested at the two-sided $\alpha = 0.05$ level.

If, in addition to a statistically significant test of the difference in the mean change from baseline corneal fluorescein staining (NEI scale) total score at Day 29 in favor of CyclASol, the test of the difference in the mean change from baseline total OSDI score at Day 29 was also statistically significant in favor of CyclASol, then CyclASol would be declared to be superior to the vehicle in both the mean change from baseline corneal fluorescein staining (NEI scale) total score and the mean change from baseline total OSDI score at Day 29.

Compliance with Good Clinical Practices

This submission was of sufficient quality to allow for a substantive review. The study was conducted according to ICHE6 Guideline for Good Clinical Practice that has its origin in the Declaration of Helsinki. No issues related to data quality or data integrity were identified in this review.

Patient Disposition

Nine investigative sites screened 727 subjects, enrolling 328 subjects at Visit 1. Of the 399 subjects not enrolled in the study, 329 failed to meet each inclusion criterion and 70 met at least one exclusion criterion.

Summary of Subject Disposition

	CyclASol 0.1% (N=162) n (%)	Vehicle (N=166) n (%)
Subjects Completing the Study		
Completed Visit 3 (Day 29 ± 2)	157 (96.9)	165 (99.4)
Completed Study	155 (95.7)	163 (98.2)
Discontinued	7 (4.3)	3 (1.8)
Subjects with Protocol Deviations		
Any protocol deviation	68 (42.0)	77 (46.4)
Major deviation	3 (1.9)	2 (1.2)
Minor deviation	68 (42.0)	76 (45.8)
Reason for Discontinuation[1]		
Adverse Events	2 (1.2)	0
Administrative Reasons	2 (1.2)	2 (1.2)
Subject Choice	3 (1.9)[2]	0
Other	0	1 (0.6)

Sources: Table 14.1.1; Listings 16.2.1,16.2.2, 16.2.3.1.
1 Percentages are based on the total number of subjects in the respective treatment group.
2 Initially, Subject (b) (6) discontinued the study drug due to an adverse event. The subject withdrew afterwards from the study by subject choice
Note: N in the headers represents the total number of subjects randomized in each respective treatment group. Percentages are based on the total number of randomized subjects (as randomized) for the denominator in the FAS. Percentages are based on the number of randomized subjects (as treated) for the denominator in the PPS and SAF.

Baseline Demographic

Demographic Information by Treatment Groups – FAS Population

	CyclASol 0.1% (N=162)	Vehicle (N=166)
Age (years)		
Mean (SD)	61.5 (13.60)	61.3 (12.66)
Median	62.0	63.0
Min, Max	18, 93	19, 89
< 45 years (n [%])	14 (8.6)	18 (10.8)
≥ 45 years < 65 years (n [%])	85 (52.5)	73 (44.0)
≥ 65 years (n [%])	63 (38.9)	75 (45.2)
Sex		
Male (n [%])	46 (28.4)	47 (28.3)
Female (n [%])	116 (71.6)	119 (71.7)
Ethnicity		
Hispanic or Latino (n [%])	15 (9.3%)	11 (6.6)
Not Hispanic or Latino (n [%])	147 (90.7%)	155 (93.4)
Race		
American Indian or Alaskan Native (n [%])	1 (0.6)	1 (0.6)
Asian (n [%])	14 (8.6)	19 (11.4)
Black or African American (n [%])	16 (9.9)	14 (8.4)
Native Hawaiian or Other Pacific Islander (n [%])	1 (0.6)	1 (0.6)
White (n [%])	130 (80.2)	127 (76.5)
Other (n [%])	0	1 (0.6)
Multiple races (n [%])	0	3 (1.8)
Iris Color (OD)		
Black (n [%])	0	3 (1.8)
Blue (n [%])	50 (30.9)	37 (22.3)
Brown (n [%])	72 (44.4)	80 (48.2)
Hazel (n [%])	23 (14.2)	34 (20.5)
Green (n [%])	16 (9.9)	10 (6.0)
Gray (n [%])	1 (0.6)	1 (0.6)

Note: N in the headers represents the number of subjects enrolled in each respective treatment group within the FAS population. All other percentages are based on the total number of subjects in each treatment group.
Sources: Table 14.1.2.1; Listings 16.2.4.1, 16.2.4.2, 16.2.4.3.

Protocol Violations/Deviations

A total of 227 protocol deviations were reported in 145 (44.2%) subjects: 107 deviations in 68 (42.0%) subjects in the CyclASol 0.1% group and 120 deviations in 77 (46.4%) subjects in the vehicle group. No deviations affected the proper enrollment of subjects.

Major Protocol Deviations

Subject Number	Treatment Group	Deviation/Description
(b) (6)	Vehicle	Improper protocol procedures at site (missed, repeated, not per protocol) / Visit 3 started less than 2 hours after subject took morning dose.
	CyclASol 0.1%	Subject's non-compliance with test article/study drug / Subject had been dosing TID for 18 days prior to Visit 3.
	Vehicle	Subject's non-compliance with test article/study drug / Visit 1 was started less than 2 hours after dosing.
	CyclASol 0.1%	Improper protocol procedures at site (missed, repeated, not per protocol) / Visit 3 was started less than 2 hours after dosing.
	CyclASol 0.1%	Subject's use of prohibited concomitant medication / Subject began taking a systemic steroid (Medrol Dose pack, five day course) 3 days prior to Visit 3.

Sources: Listing 16.2.2

Data Sets Analyzed

	CyclASol 0.1% (N=162) n (%)	Vehicle (N=166) n (%)
Full Analysis Set[1] (FAS)	162	166
Per Protocol Set[1] (PPS)	152 (93.8)	161 (97.0)
Safety Set[1] (SAF)	162 (100.0)	166 (100.0)
Total number of eyes	324	332
Qualifying eyes[2]	235 (72.5)	248 (74.7)
Right Eye (OD) Only	46 (28.4)	40 (24.1)
Left Eye (OS) Only	43 (26.5)	44 (26.5)
Both Eyes (OU)	73 (45.1)	82 (49.4)

[1]FAS used treatment as randomized; PPS and SAF used treatment as treated.
[2]Percentages are based on the total number of eyes in the respective treatment group.
Note: N in the headers represents the total number of subjects randomized in each respective treatment group. Percentages are based on the total number of randomized subjects (as randomized) for the denominator in the FAS. Percentages are based on the number of randomized subjects (as treated) for the denominator in the PPS and SAF.
Sources: Table 14.1.1; Listings 16.2.1, 16.2.2, 16.2.3.1.

Baseline Disease Characteristics (Study Eye) – FAS Population

	CyclASol 0.1% (N=162)	Vehicle (N=166)
Total Corneal Fluorescein Staining		
Mean (SD)	11.5 (1.26)	11.5 (1.25)
Median	11	11
Min, Max	10, 15	10, 15
Total OSDI Score[1]		
Mean (SD)	46.9 (16.7)	47.1 (16.4)
Median	44.10	47.61
Min, Max	20.0, 93.8	20.5, 90.0
Score < 36 (n [%])	45 (27.8%)	49 (29.5%)
Score ≥ 36 (n [%])	117 (72.2%)	117 (70.5%)
Unanesthetized Schirmer's Tear Test (mm)		
Mean (SD)	5.3 (2.82)	5.1 (2.64)
Median	5	5
Min, Max	1, 10	1, 10
Central Corneal Fluorescein Staining		
Mean (SD)	2.0 (0.51)	2.0 (0.52)
Median	2	2
Min, Max	1, 3	1, 3
Inferior Corneal Fluorescein Staining		
Mean (SD)	2.6 (0.48)	2.6 (0.51)
Median	3	3
Min, Max	2, 3	1, 3
Total Lissamine Green Conjunctival Staining		
Mean (SD)	4.2 (1.6)	4.4 (1.7)
Median	4	4
Min, Max	2, 8	2, 8
InflammaDry (MMP-9) Test		
Percent Positive	43.8%	43.4%
Best-Corrected Visual Acuity (LogMAR)		
Mean (SD)	0.120 (0.1482)	0.116 (0.1409)
Median	0.100	0.100
Min, Max	-0.24, 0.60	-0.18, 0.54
Intraocular Pressure (mmHg)		
Mean (SD)	15.7 (2.6)	15.4 (2.6)
Median	16.0	15.0
Min, Max	9, 21	10, 26

[1]Assessment relates to both eyes. Abbreviation: MMP-9 = matrix metalloproteinase 9; OSDI = Ocular Surface Disease Index; SD = standard deviation. Note: N in the headers represents the total number of subjects enrolled in each respective treatment group within the FAS population. Percentages are based on the total number of subjects in each treatment group. Baseline measures are defined as the last non-missing measure prior to the administration of study drug. The study eye from each subject is an eye that meets all of the inclusion criteria and is selected based on the mean corneal fluorescein staining score at Visit 1. Sources: Table 14.1.3.1; Listings 16.2.6.1, 16.2.6.2, 16.2.6.4, 16.2.8.4.

Reviewer's Comment: *Baseline characteristics were well balanced between the treatment groups.*

6.3 ESSENCE 2 - Study CYS-004

A Phase 3, multicenter, randomized, double-masked, vehicle-controlled clinical trial to assess the efficacy and safety of topical CyclASol for the treatment of Dry Eye Disease

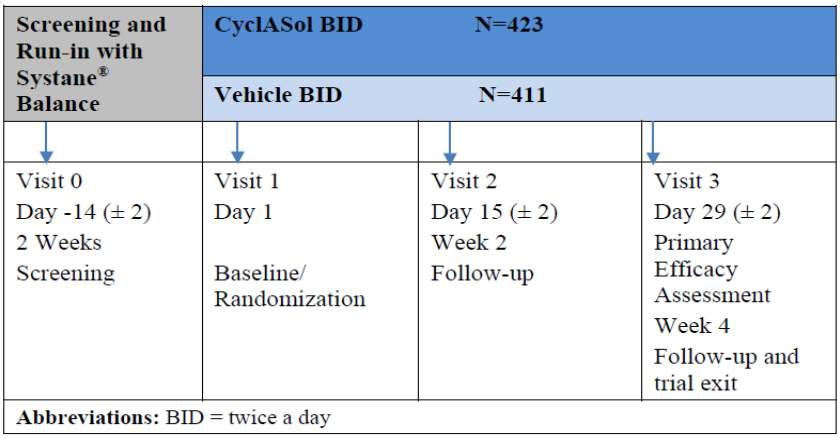
Study Design

Study CYS-004 was a Phase 3, multi-center, randomized, double-masked, vehicle-controlled clinical study to assess the efficacy and safety of topical CyclASol 0.1% for the treatment of DED. Eight hundred and thirty-four subjects of either sex and of any race who are at least 18 years of age with a subject-reported history of dry eye in both eyes and meeting all other trial eligibility criteria were randomized at 27 sites in the US to receive treatment with CyclASol or vehicle in a 1:1 ratio.

This trial was composed of two distinct parts: a run-in period and a treatment period. During the 14-day run-in period, subjects dosed with artificial tears, Systane® Balance bilaterally BID. Eligible subjects thereafter dosed the IMP (CyclASol or vehicle) bilaterally BID for approximately 29 days.

Randomized and dosed subjects who discontinued treatment for any reason were encouraged to undergo all subsequent trial visits and assessments. Data from subjects discontinuing after randomization were captured completely in the electronic case report form (eCRF) including Early Termination Visit and reason for discontinuation. If a randomized subject discontinued from the trial before Visit 3 (Day 29 ± 2 days) and was not willing to perform all subsequent visits and assessments, all evaluations of Visit 3 (at least all safety related evaluations) were performed at Early Termination Visit on the day of early termination/discontinuation or at the discretion of the investigator. This trial had no safety, special steering, or evaluation committees. No interim analyses were planned or performed.

Study Design of CYS-004 (ESSENCE-2)



List of Investigators
Subjects were enrolled at 27 sites in the United States.

Principal Investigator	Site Number	Number of Subjects Randomized
James Paauw, MD Piedmont Eye Center 116 Nationwide Drive Lynchburg, VA 24502	20	36
Robert Jordan, OD Andover Eye Associates 675 Paramount Drive, Suite 101 Raynham, MA 02767	21	11
Gail Torkildsen, MD Andover Eye Associates 138 Haverhill Street, Suite 104 Andover, MA 01810	22	21
Laura Periman, MD Periman Eye Institute 320 West Galer Street, Suite 201 Seattle, WA 98119	23	1
Dolivan Wyatt, MD Dolivan Wyatt, MD, LLC 8741 S. Greenwood Avenue, Suite 106 Chicago, IL 60619	24	0
David Wirta, MD Eye Research Foundation (now DBA Aesthetic Eye Care) 520 Superior Avenue, Suite 235 Newport Beach, CA 92663	25	90
Carol Aune, OD Oculus Research 4170 Fayetteville Road Raleigh, NC 27603 958 Vandora Springs Garner, NC 27529	26	37
Blair Boehmer, MD Midwest Cornea Associates 10300 North Illinois Street, Suite 1020 Carmel, IN 46290	27	62

Principal Investigator	Site Number	Number of Subjects Randomized
Daniel Zimmer, MD Scott & Christie Eyecare Associates 105 Brandt Drive, Suite 201 Cranberry Township, PA 16066	28	19
Patrick Vollmer, MD Vita Eye Clinic (now CORE, INC.) 222 N. Lafayette Street, Suite 12 Shelby, NC 28150	30	75
Jackson Lever, MD Country Hills Eye Center 875 Country Hills Drive Ogden, UT 84403	31	19
Eva Liang, MD Center for Sight 871 Coronado Center, Suite 130 Henderson, NV 89052	32	17
Jared Peterson, MD Alpine Research 124 South Fairfield RD, Suite C Layton, UT 84041	33	58
Robert Jordan, OD Adver Eye Associates 171 Service Avenue, Building 1, Suite 105 Warwick, RI 02887	34	4
Jung Dao, MD Cornea & Cataract Consultants 3815 East Bell Road, Suite 2500 Phoenix, AZ 85032	35	31
David Evans, OD Total Eye Care 6060 Primacy Parkway, Suite 200 Memphis, TN 38119	36	70
Guruprasad Pattar, MD Eye Care Institute 1536 Story Avenue Louisville, KY 40206 Butchertown Clinical Trials 205 N. Spring Street Louisville, KY 40206	37	51

Principal Investigator	Site Number	Number of Subjects Randomized
William Whitson, MD Michael Washburn Center for Ophthalmic Research 901 E. 86th Street Indianapolis, IN 46240	39	9
Lance Bergstrom, MD Bergstrom Eye Research 2607 University Drive South Fargo, ND 58103	40	7
Gary Jerkins, MD Advancing Vision Research 515 Stonecrest Parkway, Suite 210 Smyrna, TN 37167 Advancing Vision Research 875 S. Curtiswood Ln. Smyrna, TN 37204	41	27
Anthony Verachtert, OD Moyes Eye Center 5151 NW 88th Street Kansas City, MO 64154	42	5
Joseph Tauber, MD Tauber Eye Center 4400 Broadway Suite 202 Kansas City, MO 64111	43	4
Jonathon Downing, MD Premier Practice Management 420 E. Third Street, Suite 603 Los Angeles, CA 90013	45	115
Blake Simmons, OD Vision Institute 320 East Fontanero, Suite 201 Colorado Springs, CO 80907	46	14
Scott Schecter, OD Pinnacle Research Institute 2900 West Cypress Creek Road, Suite 13 Fort Lauderdale, FL 33309	47	26
Michael Sheety, MD Sierra Clinical Trials 2010 E. First Street, Suite 160 Santa Anna, CA 92705	48	17

Principal Investigator	Site Number	Number of Subjects Randomized
Gina Wesley Complete Eye Care of Medina 170 Westfalen Trail Hamel, MN 55430	49	8

Inclusion Criteria:
To be considered for entry into the trial, subjects were required to meet all of the following:

- Be at least 18 years of age;
- Provide written informed consent;
- Have a subject reported history of Dry Eye Disease in both eyes for at least 180 days before Visit 0;
- Be currently using or have used over-the-counter (OTC) eye drops, lubricating gels, nerve stimulation devices for tear productions (e.g. TrueTear) and/or artificial tears for dry eye symptoms within 30 days before Visit 0;
- Have a Dryness Score ≥ 50 (VAS) at Visit 0 and Visit 1;
- Have a tCFS score of ≥ 10 (i.e., sum of inferior, superior, central, nasal, and temporal regions) according to the NEI scale at Visit 0 and Visit 1;
- Have a total lissamine green conjunctival score (sum of temporal and nasal regions) of ≥ 2 according to the Oxford scale at Visit 0 and Visit 1;
- Have an unanesthetized Schirmer's tear test score between 1 mm and 10 mm inclusive at Visit 0 and Visit 1;
- Have at least one eye, the same eye, satisfy inclusion criteria 6, 7, and 8;
- Be able and willing to follow instructions and participate in all trial assessments and visits.

Key exclusion criteria:
Subjects were ineligible to participate in the trial if they met any of the following criteria:

- Have any clinically significant slit lamp findings at Visit 0 that require prescriptive medical treatment and/or in the opinion of the investigator may interfere with trial parameters including trauma, Stevens-Johnson Syndrome, advanced epithelial basement membrane disease.
- Have active blepharitis, meibomian gland dysfunction (MGD) or lid margin inflammation that required any topical or systemic antibiotics or topical steroids or other prescription medical treatment or treatment with hypochlorous acid wipes within the last 30 days prior to Visit 0 or will require such treatment during the trial. Any other therapy such as lid scrubs, lid wipes, warm compresses have to be stable within the last 30 days prior to Visit 0 and the subject should be willing to continue those therapies through the trial;
- Had Lipiflow procedures performed during the past 180 days prior to Visit 0;

- Have abnormal lid anatomy (e.g., incomplete eyelid closure, entropion, or ectropion) or abnormal blinking.
- Have Dry Eye Disease secondary to scarring from, for example, irradiation, alkali burns, cicatricial pemphigoid, or conjunctival goblet cell destruction (i.e., conjunctival goblet cell destruction because of vitamin A deficiency);
- Have an ocular or periocular malignancy.
- Have a corneal epithelial defect, or have in more than 2 of the 5 corneal regions > 50% confluent corneal staining;
- Have a history of herpetic keratitis.
- Have active ocular allergies or ocular allergies that may become active during the trial period.
- Have worn contact lenses within 90 days before Visit 0 or anticipate using contact lenses during the trial.
- Have a corrected visual acuity worse than or equal to LogMAR+0.7 as assessed by the Early Treatment of Diabetic Retinopathy Study (ETDRS) scale in either eye at Visit 0 or Visit 1.
- Be diagnosed with an ongoing ocular infection.
- Have had intraocular surgery or ocular laser surgery within 180 days before Visit 0 or have any planned ocular or eyelid surgeries during the trial period.
- Have active ocular or periocular rosacea or a pterygium.
- Have used any eye drops, lubricating gels, scrubs or nerve stimulation devices for tear production (e.g., TrueTear) within 2 hours before the first ophthalmic examination at Visit 0 and Visit 1.
- Have used topical cyclosporine or Lifitegrast within 60 days before Visit 0.
- Have used any topical antiglaucoma medications (including prostaglandin analogues applied to eyelid margin) within 90 days before Visit 0.
- Have received or removed a punctum plug within 90 days before Visit 0 or anticipate the implant or removal of a punctum plug during the trial.
- Have used any topical ocular or facial steroids, or serum tears or oral doxycycline, or oral tetracycline within 30 days before Visit 0.
- Have used any oral medications known to cause ocular drying (e.g., antihistamines or antidepressants) on a non-stable regimen within 30 days before Visit 0 or anticipate non-stable use of oral ocular-drying medication during the trial.
- Have used systemic steroids (including dermatological steroids with high potency or large treatment areas) or immunomodulating agents on a non-stable regimen within 90 days before Visit 0 or anticipate their use on a non-stable regimen during the trial period.
- Have an ongoing systemic infection (bacterial, viral, or fungal), including a fever, and/or requiring treatment with antibiotics at Visit 0 or Visit 1 and/or positive SARS-CoV-2 (COVID-19) test within 2 weeks before Visit 0 and until Visit 1.
- Have been randomized in a previous CyclASol trial.
- Be a woman who is pregnant, nursing, or planning a pregnancy.

- Be unwilling to submit a urine pregnancy test at Visit 0 and Visit 3 (or early termination visit) if of childbearing potential. Non-childbearing potential is defined as a woman who is permanently sterilized (i.e., has had a hysterectomy, bilateral tubal ligation, or bilateral oophorectomy), or is post-menopausal (i.e., without menses for 12 consecutive months).
- Be a woman of childbearing potential who is not using an acceptable means of contraception. Acceptable methods of contraception include hormonal contraceptives (i.e., oral, implantable, injectable, or transdermal contraceptives), mechanical contraceptives (i.e., spermicide in conjunction with a barrier such as a diaphragm or a condom), intrauterine devices (IUD), or the surgical sterilization of the partner. For non-sexually active females, abstinence may be regarded as an adequate method of birth control; however, if the subject becomes sexually active during the trial, she must agree to use adequate birth control as defined above for the remainder of the trial.
- Have an uncontrolled systemic disease;
- Have a known allergy or sensitivity to the IMP or its components: Cyclosporine or semi fluorinated alkanes (SFA).
- Currently using an active investigational drug or device or have used an investigational drug or device within 60 days before Visit 0 (potential vehicles used for run-in periods in other trials are not considered active investigational products); or
- Have a condition or be in a situation (e.g., language barrier, illiteracy) which the investigator feels may put the subject at significant risk, may confound the trial results, or may interfere with the subject's participation in the trial significantly.

Treatments Administered
Run-In

- Systane Balance Lubricant Eye Drops (Propylene Glycol 0.6%)
  At the end of Visit 0, qualified subjects received one bottle of the run-in medication (Systane Balance, Lot Number: 103WL (30-Sep-2021) for two weeks starting with an in-office dose. Subjects were instructed to dose at home twice daily (BID), in the morning and in the evening at bedtime, up to and including the morning of Visit 1 (at least 2 hours before first ophthalmic examination). Subjects recorded in the subject's diary that their doses were taken and used/unused run-in was collected from them at Visit 1.
- Randomized Study Treatments

Test Product	Dose	Supplier	Batch Number
CyclASol 0.1% ophthalmic solution	BID	Alliance Medical Products, Inc.	2039-63
Vehicle ophthalmic solution	BID	Alliance Medical Products, Inc.	2039-50

Prohibited Medications and Procedures
Disallowed medications and treatments are listed in the Exclusion Criteria (Section 9.3.2). All medications and treatments that were not allowed prior to the trial were also not allowed during the trial, in particular no other prescription or over-the-counter topical ophthalmic

medications including dry eye treatments such as artificial tears (other than run-in medication), gels, ointments or TrueTear™ device (Intranasal Tear Neurostimulator) were allowed within 2 hours prior to Visit 0 and throughout the course of the trial.
The following medications and treatments were prohibited:

- Any eye drops, lubricating gels, scrubs or nerve stimulation devices for tear production (e.g., TrueTear) within 2 hours before the first ophthalmic examination at Visit 0 and Visit 1.
- Topical cyclosporine or Lifitegrast within 60 days before Visit 0.
- Any topical antiglaucoma medications (including prostaglandin analogues applied to eyelid margin) within 90 days before Visit 0.
- Implantation or removal of a punctum plug within 90 days before Visit 0 or anticipate the implant or removal of a punctum plug during the trial.
- Any topical ocular or facial steroids, or serum tears or oral doxycycline, or oral tetracycline within 30 days before Visit 0.
- Any oral medications known to cause ocular drying (e.g., antihistamines or antidepressants) on a non-stable regimen within 30 days before Visit 0 or anticipate non-stable use of oral ocular-drying medication during the trial.
- Systemic steroids (including dermatological steroids with high potency or large treatment areas) or immunomodulating agents on a non-stable regimen within 90 days before Visit 0 or anticipate their use on a non-stable regimen during the trial period.
- Antibiotics at Visit 0 or Visit 1 for the treatment of any infections.
- Currently using an active investigational drug or device or have used an investigational drug or device within 60 days before Visit 0 (potential vehicles used for run-in periods in other trials are not considered active investigational products); or Hypochlorous acid wipes.

Rescue Medication
There were no rescue medications used in this trial.

Criteria for Evaluation

Primary Efficacy Measures

- Change from baseline (CFB) in total corneal fluorescein staining score (tCFS, NEI scale) in the study eye at Day 29
- CFB in Dryness Score (VAS) at Day 29

Key Secondary Efficacy Measures

- CFB in total conjunctival lissamine green staining score (Oxford scale) at Day 29
- Proportion of responders in central corneal fluorescein staining (cCFS) score (≥ 1 score improvement on NEI scale) at Day 29
- Proportion of responders in tCFS score (≥ 3 score improvement on NEI scale) at Day 29
- CFB in cCFS score (NEI scale) to Day 29
- CFB in tCFS score (NEI scale) at Day 15
- CFB in blurred vision (VAS) at Day 29

Secondary Efficacy Measures

- cCFS score and CFB at Day 15
- Total conjunctival lissamine green staining score and by region (Oxford scale) and CFB to each measured post-baseline visit (except CFB in total conjunctival lissamine staining at Day 29)
- Dryness Score and blurred vision VAS at each post-baseline visit and CFB to Day 15

Exploratory Efficacy Measures:

- Reading assessments (MNRead; sustained silent reading test; IReST), and CFB at each measured post-baseline visit
- CFS score per sub-regions (other than cCFS; NEI scale) and CFB at each measured post-baseline visit
- Proportion of responders in tCFS score (≥ 2 and ≥ 4 scores improvement on NEI scale) at Day 15 and Day 29
- VAS for frequency of dryness, awareness of dryness, reading problems, fluctuating vision, looking at screens and driving and CFB at night at each measured post-baseline visit
- Ocular Surface Disease Index (OSDI©) total, individual and subtotal scores and CFB at each measured post-baseline visit
- Unanesthetized Schirmer's tear test and CFB to each measured post-baseline visit
- Proportion of responders in unanesthetized Schirmer's tear test (≥ 10 mm increase) at each measured post-baseline visit
- Tear film break-up time (TFBUT) and CFB at each measured post-baseline visit.

Statistical Analysis Plan
Analysis populations were:

- The Full Analysis Set (FAS) – Same as Study CYS-002
- The Per Protocol Set (PPS) – Same as Study CYS-002
- The Safety Set (SAF) – Same as Study CYS-002

Primary Efficacy Analysis

The primary comparisons in this trial were between CyclASol versus its vehicle at Day 29 in the FAS with available data. The primary efficacy analysis compared the mean changes from baseline in total corneal fluorescein staining (NEI Scale) in the study eye and dryness score (VAS), which were analyzed separately using an ANCOVA model with terms for baseline value, site and treatment group. Least squares mean for each treatment group and for the difference between treatment groups were presented from the model together with two-sided p- values and 95% confidence intervals.

Safety Analysis

All safety assessments were analyzed with the SAF (safety set) and subject listing of all safety parameters were produced. Adverse events (AEs) were coded using the Medical Dictionary

for Regulatory Activities (MedDRA) dictionary. Frequencies and percentages of treatment-emergent adverse events (TEAEs) were summarized at the subject level by system organ class and preferred term for all TEAEs, treatment related TEAEs, treatment emergent SAEs, and TEAEs causing premature discontinuation by treatment group. An AE was treatment emergent if it occurred or worsened after the first dose of trial treatment. Similar summaries were presented for all TEAEs by maximal severity. Separate summaries were performed for ocular and non-ocular AEs.

Other safety endpoints including visual acuity, slit lamp biomicroscopy, intraocular pressure (IOP) and dilated fundoscopy were summarized by treatment group and visit using descriptive statistics. Changes or shifts from baseline were summarized where appropriate. Assessments performed by eye, study eye and fellow eye were summarized separately.

Schedule of Visits and Assessments

Procedure	Visit 0 Day -14 ± 2	Visit 1 Day 1	Visit 2 Day 15 ± 2	Visit 3 Day 29 ± 2
Informed Consent / HIPAA	X			
Medical / Medication History and Demographics	X			
Review of Qualification Criteria	X	X		
Urine Pregnancy Test (as needed)[1]	X			X
Medical/Medication Update		X	X	X
Recording of Adverse Events	X	X	X	X
Dryness Score (VAS)	X	X	X	X
VAS (frequency of dryness, awareness of dryness symptoms, blurred vision, reading problems, fluctuating vision, looking at screens, driving at night)	X	X	X	X
Ocular Surface and Disease Index (OSDI)	X	X	X	X
Visual Acuity (ETDRS)	X	X	X	X
Slit lamp Biomicroscopy	X	X	X	X
Tear Film Break-Up Time	X	X		X
Fluorescein Staining - NEI Scale	X	X	X	X
Lissamine Green Staining - Oxford Scale	X	X	X	X
Reading Assessments (MNRead, SSRT, IReST)		X		X
Schirmer's Tear Test I (without anaesthesia)	X	X		X
Intraocular Pressure	X			X
Dilated Fundoscopy	X			X

In-office instillation of run-in	X			
Run-in & Diary Dispensation	X			
Run-in Collection		X		
Randomization		X		
In-office instillation of randomized study drug		X	X	
Drop Comfort Scale and Questionnaire		X	X	
Eyedrop Acceptability Questionnaire				X
Dispensation of study medication		X	X	
Collection of study medication			X	X
Diary review/collection		X	X	X
Trial Exit				X

[1]For women of childbearing potential
Abbreviations: ETDRS = Early Treatment Diabetic Retinopathy Study, HIPAA = Health Insurance Portability and Accountability Act, IReST= International Reading Speed Test, NEI = National Eye Institute, MNRead = Minnesota low vision reading chart , OSDI = Ocular Surface and Disease Index, SSRT= Sustained Silent Reading Test VAS=Visual Analog Scale

Compliance with Good Clinical Practices

This submission was of sufficient quality to allow for a substantive review. The study was conducted according to ICH-E6 Guideline for Good Clinical Practice that has its origin in the Declaration of Helsinki. No issues related to data quality or data integrity were identified in this review.

Patient Disposition

Overall, 1,879 subjects were screened for the trial. 834 were enrolled in the trial and randomized. 817 subjects (98.0%) completed the trial and 17 subjects (2.0%) discontinued.

The 17 subjects (2%) that discontinued the trial were balanced between treatment arms and the main reasons for discontinuation were subject choice (10 subjects), lost to follow- up (3 subjects), adverse events (2 subjects), and other reasons: investigator decision (1 subject) and pregnancy (1 subject).

Subject Disposition

	CyclASol (N=423) n (%)	Vehicle (N=411) n (%)
Number of Eyes		
Total Number of Eyes	846	822
Number of Qualifying Eyes[2]	648 (76.6%)	624 (75.9%)
Right Eye (OD Only	92 (10.9%)	86 (10.5%)

Left Eye (OS) Only	106 (12.5%)	112 (13.6%)
Both Eyes (OU)	450 (53.2%)	426 (51.8%)
Trial Completion		
Completed	415 (98.1%)	402 (97.8%)
Discontinued	8 (1.9%)	9 (2.2%)
Discontinuations Related to COVID-19	0	0
Reason for Discontinuation[3]		
Adverse Event	2 (0.5%)	0
Protocol Violation	0	0
Lack of Efficacy	0	0
Administrative Reasons	0	0
Lost to Follow Up	0	3 (0.8%)
Sponsor Termination of Trial	0	0
Subject Choice	5 (1.2%)	5 (1.2%)
Other	1 (0.2%)	1 (0.2%)

Note: N in the headers represents the total number of subjects randomized in each respective treatment group. Percentages are based on the total number of subjects randomized in each treatment group.
[1]For the treatment assignments, Full Analysis Set includes subjects as randomized, while Safety Set and Per Protocol Set include subjects as treated.
[2]Percentages are based on the total number of eyes in the respective treatment group.
[3]Percentages are based on the total number of subjects in treatment group.
[4]Severities of the deviations were assigned by the sponsor prior to database lock and unmasking.
[5]Subjects with multiple deviations are only counted once in each category.
Sources: Module 5.3.5.1 CYS-004 CSR, Table 4 (Table 14.1.1, Listings 16.2.1.1, 16.2.2, 16.2.3.1, 16.2.3.3)

Protocol Violations/Deviations

Protocol deviations were classified as major or minor by the sponsor and the contract research organization (CRO) before database lock and unmasking.
The key types of protocol deviations included visit out of window (11.3%), improper protocol procedures at site (9.4%), informed consent (6.8%) and subject's non-compliance with test article/study drug (4.0%). The protocol deviations related to the informed consent were all related to re-consenting, which was necessary due to minor updates in the informed consent form.

Data Sets Analyzed

Study CYS-004 Analysis Populations (Randomized Set)

	CyclASol (N=423) n (%)	Vehicle (N=411) n (%)
Analysis Populations[1]		

Full Analysis Set	423 (100.0%)	411 (100.0%)
Per Protocol Set	405 (95.7%)	391 (95.1%)
Safety Set	423 (100.0%)	411 (100.0%)

Source: Module 5.3.5.1 CYS-004 CSR Table 4

Demographics – Full Analysis Set Population

	CyclASol (N=423)	Vehicle (N=411)
Age (Years)		
Mean (SD)	57.6 (15.36)	56.6 (16.30)
Median	60.0	59.0
Min, Max	19, 86	18, 93
Age Category: n (%)		
< 45 Years	85 (20.1%)	90 (21.9%)
>= 45 and < 65 Years	181 (42.8%)	171 (41.6%)
>= 65 Years	157 (37.1%)	150 (36.5%)
Sex: n (%)		
Male	117 (27.7%)	108 (26.3%)
Female	306 (72.3%)	303 (73.7%)
Childbearing potential[1]	59 (19.3%)	72 (23.8%)
Ethnicity: n (%)		
Hispanic or Latino	62 (14.7%)	60 (14.6%)
Not Hispanic or Latino	359 (84.9%)	348 (84.7%)
Unknown	1 (0.2%)	0
Not Reported	1 (0.2%)	3 (0.7%)
Race: n (%)		
American Indian or Alaska Native	2 (0.5%)	0
Asian	40 (9.5%)	39 (9.5%)
Black or African American	53 (12.5%)	55 (13.4%)
Native Hawaiian or Other Pacific Islander	1 (0.2%)	0
White	323 (76.4%)	312 (75.9%)
Other	1 (0.2%)	1 (0.2%)
Multiple	2 (0.5%)	3 (0.7%)
Unknown	0	1 (0.2%)
Not Reported	1 (0.2%)	0

	CyclASol (N=423)	Vehicle (N=411)
Dry Eye Disease Duration (years)		
Mean (SD)	10.3 (9.55)	10.4 (10.32)
Median	6.4	6.3
Min, Max	1, 65	1, 55
Dry Eye Disease Duration Category: n (%) < 10 years	261 (61.7%)	256 (62.3%)
>= 10 years	162 (38.3%)	155 (37.7%)

Source: Module 5.3.5.1 CSR CYS-004, Table 5

Reviewer's Comment: *Overall, the treatment groups were well balanced regarding demographic characteristics.*

Baseline Disease Characteristics – Full Analyses Set Population

Measurement	CyclASol (N=423)	Vehicle (N=411)
Primary Endpoints		
Total Corneal Fluorescein Staining (0 - 15: Higher is Worse)		
Mean (SD)	11.5 (1.41)	11.5 (1.36)
Median	11.0	11.0
Min, Max	10, 15	8, 15
Dryness from VAS (0 - 100: Higher is Worse)		
Mean (SD)	70.4 (12.53)	70.0 (12.59)
Median	70.0	70.0
Min, Max	50, 100	50, 100
Total Conjunctival Lissamine Green Staining (0 – 10: Higher is Worse)		
Mean (SD)	3.7 (1.72)	3.8 (1.67)
Median	3.0	3.0
Min, Max	2, 10	2, 8
Central Corneal Fluorescein Staining (0 – 3: Higher is Worse)		
Mean (SD)	2.1 (0.63)	2.1 (0.63)
Median	2.0	2.0
Min, Max	1, 3	1, 3
Blurred Vision from VAS (0 – 100: Higher is Worse)		
Mean (SD)	53.4 (26.56)	51.9 (24.97)
Median	59.0	57.0

Min, Max	0, 100	0, 100
Total Ocular Surface Disease Index (0 -100: Higher is Worse)		
Mean (SD)	47.06 (21.066)	47.15 (19.290)
Median	47.50	47.50
Min, Max	0.0, 100.0	0.0, 93.2
Unanesthetized Schirmer's Tear Test (mm)		
Mean (SD)	5.1 (2.96)	4.8 (2.78)
Median	5.0	4.0
Min, Max	1, 10	1, 10
Tear Film Break-Up Time (seconds)		
Mean (SD)	3.3555 (1.45211)	3.2793 (1.56159)
Median	2.9800	2.8550
Min, Max	0.730, 10.200	1.020, 17.205
Visual Acuity (LogMAR)		
Mean (SD)	0.110 (0.1674)	0.113 (0.1641)
Median	0.100	0.100
Min, Max	-0.20, 0.68	-0.30, 0.72
Intraocular Pressure (mmHg)		
Mean (SD)	15.6 (2.88)	15.5 (2.95)
Median	16.0	16.0
Min, Max	8, 23	6, 26
Dry Eye Disease Duration (years)		
N	423	411
Mean (SD)	10.3 (9.55)	10.4 (10.32)
Median	6.4	6.3
Min, Max	1, 65	1, 55
Dry Eye Disease Duration Category: n (%) < 10 years	261 (61.7%)	256 (62.3%)
>= 10 years	162 (38.3%)	155 (37.7%)

Abbreviations: LogMAR = Logarithm of the Minimum Angle of Resolution; mmHg = millimeters of Mercury; NEI = National EyeInstitute/Industry; tCFS = Total Corneal Fluorescein Staining; VAS = Visual Analog Scale. Note: N in the headers represents the total number of subjects in the given treatment for the population being analyzed. Study eye was the eye with higher mean tCFS score (NEI Scale) at Visit 1 (Day 1) if both eyes were eligible. If the mean tCFS score was equivalent in both eyes, then the right eye was selected as the study eye. All reported endpoints are for study eye or subject level unless otherwise noted. Baseline measures were defined as the last non-missing measure prior to the initiation of randomized study treatment, usually at Visit 1 (Day 1).

References: Table 14.1.3.1, Listings 16.2.6.1, 16.2.6.2, 16.2.6.3, 16.2.6.7, 16.2.6.8, 16.2.6.9, 16.2.8.1,

Reviewer's Comment: *Overall, the treatment groups were well balanced regarding baseline disease characteristics.*

7. Integrated Review of Effectiveness

Studies CYS-002, CYS-003 and CYS-004 were randomized, double-masked, vehicle-controlled and multi-center clinical studies conducted in the US in subjects with dry eye disease (DED). They were conducted sequentially of similar in design except for the study duration, the study size and the primary symptom endpoint. Study CYS-002 was conducted in 2016, Study CYS-003 in 2017/ 2018, and study CYS-004 in 2020/ 2021. Overall, the studies had similar eligibility criteria for DED subjects, the same CyclASol concentration, dose and dose regimen and the same visit schedule up to Visit 3 (Day 29). The studies differed with regard to the primary symptom efficacy endpoint. CYS-003 and CYS-004 consisted of two periods: a 14-day run-in period and then a treatment period. During the 14-day run-in period, subjects self-administered Systane Balance Lubricant Eye Drops bilaterally twice daily (BID). In Study CYS-003, 328 subjects were randomized in a 1:1 ratio to receive CyclASol 0.1% or the vehicle bilaterally BID for three months with the study visits on Days -14 (screening), 1 (baseline/randomization), 15, 29, 57, and 85. In Study CYS-004, 834 subjects were randomized in a 1:1 ratio to receive CyclASol 0.1% or the vehicle bilaterally BID for one month with the study visits on Days -14 (screening), 1 (baseline/randomization), 15, and 29.

Demonstration of a 10mm or greater improvement from baseline in Schirmer's tear test has been the basis of support for other cyclosporine containing topical ophthalmic products which have been approved in the United States. Studies CYS-002 and CYS-004 demonstrated superiority of CyclASol 0.1% to its vehicle on the proportion of responders in Schirmer's tear test at Day 29. Study CYS-003 did not demonstrate superiority in the proportion of responders at Day 29, but did on Day 85.

As originally designed, the primary sign endpoint, for each study was the change from baseline in tCFS score. The endpoint day was specified as Day 29 for CYS-003 and CYS-004, but was not specified for Study CYS-002. Studies CYS-003 and CYS-004 demonstrated statistical superiority of CyclASol 0.1% over its vehicle in tCFS, however since clinical superiority requires a demonstration of a change in symptoms, neither study was considered to have demonstrated clinical superiority for this endpoint.

Analysis of primary endpoints: change from baseline in total corneal fluorescein staining (NEI scale), ocular surface disease index and dryness score at Day 29 (FAS) – CYS-002, CYS-003, CYS-004

	CYS-002				CYS-003		CYS-004	
	CyclASol 0.05% (N=51)	CyclASol 0.1% (N=51)	Restasis (N=53)	Vehicle (N=52)	CyclASol 0.1% (N=162)	Vehicle (N=166)	CyclASol 0.1% (N=423)	Vehicle (N=411)
Sign endpoint- Total Corneal Fluorescein Staining (NEI scale) Scale								
Baseline								
N	51	51	53	52	162	166	423	411
Mean (SD)	8.8 (2.1)	8.8 (1.9)	8.8 (1.9)	8.7 (2.1)	11.5 (1.3)	11.5 (1.3)	11.5 (1.4)	11.5 (1.4)
Visit 3 (Day 29)								
N	50	51	52	50	157	165	409	395
Mean (SD)	6.9 (2.5)	6.9 (2.0)	8.0 (2.5)	7.3 (2.6)	8.5 (2.77)	9.3 (2.82)	7.1 (3.34)	7.6 (3.60)
Change from baseline to Day 29								
Mean (SD)	-1.9 (2.1)	-1.9 (2.0)	-0.8 (2.5)	-1.4 (2.3)	-2.9 (2.6)	-2.2 (2.7)	-4.3 (3.2)	-3.9 (3.4)
ANCOVA LS mean					-3.5	-2.6	-4.0	-3.6
Two-sided 95% CI					(-3.8, -3.2)	(-3.0, -2.3)	(-4.3, -3.7)	(-3.9, -3.3)
LS mean difference*	-0.42	-0.48			-0.8		-0.41	
Two-sided 95% CI	(-1.4,0.6)	(-1.4,0.4)			(-1.3, -0.4)		(-0.8, -0.04)	
p-value	0.4128	0.3111			0.0002		0.0278	
Visit 5 (Day 113)								
N	50	50		48				
Mean (SD)	6.5	6.6		7.1				
Difference from Vehicle	-0.6	-0.5						
p-value (paired t-test)	0.2818	0.4235						
Change from baseline to Day 113	-2.4	-2.1	-1.6	-1.7				
Two-sided 95% CI	-3.0,-1.8	-2.7,-1.5						
LS mean difference*	-0.7	-0.4	0.14					
Two-sided 95% CI	-1.6,0.2	-1.3,0.5	-0.7,1.0					
p-value	0.1334	0.3505						
Symptom endpoints- Ocular Surface Disease Index								
Baseline								
N	51	51	53	52	162	166	423	411
Mean (SD)	38.0	40.4	35.4	37.9	46.9 (16.7)	47.1 (16.4)	47.1 (16.4)	47.2 (19.3)
Visit 3 (Day 29)								
N					157	165		
Mean (SD)	35.1	38.3	27.6	37.5	39.8 (19.6)	41.9 (19.1)		
Change from baseline to Day 29								
N					157	165		
Mean (SD)	-3.2	-2.1	-7.8	-0.6	-7.1 (18.6)	-5.4 (15.3)	-6.0 (16.9)	-7.2 (17.6)
ANCOVA LS mean					-8.8	-6.8	-6.1	-7.4
Two-sided 95% CI					(-11.4, -6.1)	(-9.3, -4.3)		
LS mean difference*					-1.95		1.2	
Two-sided 95% CI					(-5.4, 1.5)		(-1.0,3.5)	
p-value					0.2634		0.2791	

Visit 5 (Day 113)								
N								
Mean (SD)								
Change from baseline to Day 113								
N	51	51	53	52				
Mean (SD)	-11.0	-9.3	-13.9	-19.0				
ANCOVA LS mean								
LS mean difference*	2.5	4.3	-5.5					
Two-sided 95% CI	-7.2,12.1	-5.4,13.9	-15.1,4.0					
Symptom endpoints- Dryness Score								
Baseline								
N	51	51	53	52	162	166	423	411
Mean (SD)	72.8 (19.6)	71.2 (16.2)	68.5 (18.8)	68.5 (21.4)	68.5 (21.7)	69.9 (20.5)	70.4 (12.5)	70.0 (12.6)
Visit 3 (Day 29)								
N							409	395
Mean (SD)	53.8 (19.8)	58.2 (21.7)	50.8 (22.3)	59.7 (19.2)			57.9 (24.53)	56.2 (24.91)
Change from baseline to Day 29								
N					157	165	409	395
Mean (SD)	-9.8 (17.8)	-6.3 (22.0)	-12.9 (19.1)	-5.1 (17.8)	-9.3 (22.2)	-5.4 (20.6)	-12.6 (23.72)	-13.7 (23.41)
ANCOVA LS mean					-10.7 (1.7)	-5.9 (1.6)	-12.2	-13.6
Two-sided 95% CI							(-14.7, -9.7)	(-16.2, -11.0)
LS mean difference*					-4.8 (2.2)		1.4 (1.64)	
Two-sided 95% CI					(-9.1,-0.4)		(-1.8, 4.6)	
p-value					0.0311		0.3842	
Visit 5 (Day 113)								
N	50	50		48				
Mean (SD)	52.7	54.8		51.2				
Difference from Vehicle	1.5	3.6						
p-value (paired t-test)	0.7648	0.5098						
Change from baseline to Day 113	-11.0	-9.3		-13.8				
Two-sided 95% CI	-16.5,-5.5	-17.2,-1.4						
LS mean difference*	2.5	4.3						
Two-sided 95% CI	-7.2,12.1	-5.4,13.9						
p-value	0.6166	0.3829						

ANCOVA=analysis of covariance, CI=confidence interval, FAS=full analysis set, LS=least squares, N=number of subjects in group, NEI=National Eye Institute, OSDI=ocular surface disease index, SD=standard deviation, tCFS=total corneal fluorescein staining. *CyclASol-Vehicle. Note: N in headers represented the total number of subjects enrolled in each respective treatment group within the FAS population. Note for tCFS analyses: Analyses were for the study eye. The study eye from each subject was the eye that met all of the inclusion criteria and was selected based on the mean fluorescein corneal staining score t Visit 1. Corneal fluorescein staining was assessed on a 0-3 scale in each of five regions of the cornea. A score of 0 meant no staining and 3 meant severe staining. Total corneal fluorescein staining was the sum of staining scores for all five regions, on a 0-15 scale. ANCOVA model included site and treatment as fixed factors, and baseline score as a covariate. (CYS-003), and baseline value, site and treatment group (CYS-004). Note for OSDI analyses: Analyses were for both eyes. ANCOVA model included site and treatment as fixed factors, and baseline score as a covariate.

Reviewer's Comment: *Both Study CYS-003 and Study CYS-004 demonstrated a statistically significant treatment group difference favoring CyclASol 0.1% in the change from baseline in total*

corneal fluorescein staining at Day 29. The change was not considered to be clinically significant because neither Study CYS-003 nor CYS-004 demonstrated a statistically significant treatment group difference in the sign (Ocular Surface Disease Index (OSDI) at Day 29 or eye dryness score at Day 29, respectively).

At the Agency's request, the applicant submitted an analysis for the proportion of subjects with a greater than or equal to 10 mm increase in unanesthetized Schirmer's tear test. This endpoint was not the primary endpoint in either study, but is the endpoint used to evaluate other topical ophthalmic products.

Results for Proportion of Schirmer's Tear Test Responders, Using FAS with Available Data

		CYS-002		CYS-003		CYS-004	
		CyclASol 0.1% (N=51)	Vehicle (N=52)	CyclASol 0.1% (N=162)	Vehicle (N=166)	CyclASol 0.1% (N=423)	Vehicle (N=411)
Day 15	N	51	51	160	164		
	Responders, n (%)	1 (1.96%)	0 (0.00%)	13 (8.12%)	6 (3.66%)		
	Difference (95% CI) [1]	1.96% (-1.84%, 5.77%)		4.47% (-0.65%, 9.58%)			
	p-value[1]	0.3149		0.0871			
	Odds Ratio (95% CI)[2]	-		2.33 (0.90, 6.78)			
	p-value[2]	-		0.0952			
	p-value[3]	1		0.1012			
Day 29	N	51	50	157	165	409	395
	Responders, n (%)	4 (7.84%)	0 (0.00%)	7 (4.46 %)	7 (4.24 %)	44 (10.76%)	27 (6.84%)
	Difference (95% CI) [1]	7.84% (0.46%, 15.22%)		0.22% (-4.24%, 4.67%)		3.92 % (0.02%, 7.82%)	
	p-value[1]	0.0433		0.9243		0.0500	
	Odds Ratio (95% CI)[2]	-		1.05 (0.35, 3.15)		1.64 (1.00, 2.74)	
	p-value[2]	-		0.9243		0.0519	
	p-value[3]	0.1176		1		0.0617	
Day 57	N			156	164		
	Responders, n (%)			9 (5.77%)	7 (4.27%)		
	Difference (95% CI) [1]			1.50% (-3.29%, 6.29%)			
	p-value[1]			0.5380			
	Odds Ratio (95% CI)[2]			1.37 (0.50, 3.93)			
	p-value[2]			0.5395			
	p-value[3]			0.6131			
Day 85	N	49	50	155	163		
	Responders, n (%)	2 (4.08%)	5 (10.00%)	19 (12.26%)	9 (5.52%)		
	Difference (95% CI) [1]	-5.92% (-15.91%, 4.07%)		6.74% (0.50%, 12.98%)			
	p-value[1]	0.2507		0.0341			
	Odds Ratio (95% CI)[2]	0.38 (0.05, 1.88)		2.39 (1.07, 5.71)			
	p-value[2]	0.2656		0.0386			
	p-value[3]	0.4360		0.0465			
Day 113	N	50	48				
	Responders, n (%)	3 (6.00%)	3 (6.25%)				
	Difference (95% CI) [1]	-0.25% (-9.75%, 9.25%)					
	p-value[1]	0.9588					
	Odds Ratio (95% CI)[2]	0.96 (0.17, 5.41)					
	p-value[2]	0.9588					
	p-value[3]	1					

[1] The 95% CI and p-value are based on Normal approximation.

[2] The odds ratio, 95% CI and p-value are calculated using logistic regression model with a factor for treatment group.
[3] The p-value is based on Fisher's exact test.

8. Review of Safety

8.1. Safety Review Approach

The Applicant's primary support for safety for Vevye (cyclosporine ophthalmic solution) 0.1 % for the treatment of dry eye disease is based on data from ESSENCE-1 (CYS-003) AND ESSENCE-2 (CYS-004). This review evaluates the Pool A safety database presented in the application. Pool A consist of the safety populations in Study CYS-003 and CYS-004.

8.2. Review of the Safety Database

8.2.1. Overall Exposure

Extent of treatment exposure (SAF) – (CYS-003 + CYS-004)

Treatment Exposure (days)	CyclOl 0.1% (N=585)	Vehicle (N=577)
Mean (SD)	43.9 (25.09)	44.8 (25.69)
Median (range)	29.0 (3, 98)	29.0 (1, 117)
Total	25670	25860
Exposure Duration: n (%)		
0 – 14 days	4 (0.7%)	3 (0.5%)
15 – 29 days	344 (58.8%)	342 (59.3%)
30 – 85 days	180 (30.8%)	173 (30.0%)
86 – 183 days	57 (9.7%)	59 (10.2%)

N=number of subjects in group, SAF=safety set, SD=standard deviation.
Note: Percentages are based on the total number of subjects in each treatment group and integration pool. Subjects who participated in CYS-005 and who received vehicle in CYS-004 are counted in both the CyclASol 0.1% and Vehicle treatment arms. Source: ISS Table 14.3.7.1.

Reviewer's Comment: *The treatment exposure to Vevye (cyclosporine ophthalmic solution) 0.1% was sufficient to assess safety.*

8.2.2. Relevant characteristics of the safety population:

The safety population is representative of the population that cyclosporine is intended to treat for the dry eye disease indication.

8.2.3. Adequacy of the safety database:

The safety database is adequate with respect to size, duration of exposure, duration of treatment, patient demographics, and disease characteristics.

8.3. Safety Results

8.3.1. Deaths

There were no deaths during clinical studies, CYS-003 and CYS-004.

8.3.2. Dropouts and/or Discontinuations Due to Adverse Effects

Treatment-emergent adverse events leading to treatment discontinuation (SAF) (CYS-003 + CYS-004)

System Organ Class (SOC)	CyclASol 0.1% (N=585)	Vehicle (N=577)
Preferred Term (PT)	n (%)	n (%)
Number of TEAEs Leading to Treatment Discontinuation	5	5
Number of Subjects with at Least One TEAE Leading to Treatment Discontinuation	5 (0.9%)	3 (0.5%)
Eye disorders	3 (0.5%)	1 (0.2%)
Foreign body sensation in eyes	1 (0.2%)	1 (0.2%)
Eye irritation	0	1 (0.2%)
Eye pain	0	0
Ocular discomfort	1 (0.2%)	0
Photophobia	0	1 (0.2%)
Swelling of eyelid	1 (0.2%)	0
General disorders and administration site conditions	1 (0.2%)	1 (0.2%)
Instillation site pain	1 (0.2%)	1 (0.2%)
Injury, poisoning and procedural complications	0	1 (0.2%)
Chemical burns of eye	0	0
Exposure during pregnancy	0	1 (0.2%)
Maternal exposure during pregnancy	0	0
Hepatobiliary disorders	1 (0.2%)	0
Cholelithiasis	1 (0.2%)	0
Nervous system disorders	0	0
Dysgeusia	0	0

Note: Subjects who participated in CYS-005 and who received vehicle in CYS-004 are counted in both the CyclASol 0.1% and Vehicle treatment arms. Percentages are based on the total number of subjects in each treatment group and integration pool.
TEAEs are AEs that started or worsened on or after the date of first dose.
SOCs and PTs within a SOC are listed in order of descending frequency for All Subjects in the Pool B population. Subjects experiencing more than one TEAE within a given SOC or PT are counted once within that SOC or PT.
Source: ISS Table 14.3.1.13.1.

Reviewer's Comment: *Treatment discontinuations due to adverse events were balanced between treatment groups and did not suggest any safety signals. No adverse event which led to treatment discontinuation occurred in ≥ 1% of subjects.*

Five subjects in the CyclASol and Vehicle groups discontinued study treatment due to an ocular adverse event consistent with dry eye disease. Two subjects discontinued due to non-ocular adverse events.

8.3.3. Significant Adverse Events

Serious adverse events (SAF) – Pool A (CYS-003 + CYS-004)

System Organ Class (SOC) Preferred Term (PT)	CyclASol 0.1% (N=585) n (%)	Vehicle (N=577) n (%)
Number of Subjects with at Least One SAE	2 (0.3%)	6 (1.0%)
Pneumonia	0	1 (0.2%)
Appendicitis	0	1 (0.2%)
Localized infection	1 (0.2%)	0
Ophthalmic herpes simplex	0	1 (0.2%)
Intestinal obstruction	0	1 (0.2%)
Small intestinal obstruction	0	1 (0.2%)
Cholelithiasis	1 (0.2%)	0
Intervertebral disc degeneration	0	1 (0.2%)

Reviewer's Comment: *The incidence of serious adverse events was higher in the vehicle treatment group. The only ocular serious adverse event was a case of ocular herpes simplex keratitis in the vehicle group in Study CYS-004. The reported non-ocular serious adverse events were generally unrelated.*

Common Adverse Events (in > 2 subjects) (SAF) (CYS-003 + CYS-004)

System Organ Class (SOC) Preferred Term (PT)	CyclASol 0.1% (N=585) n (%)	Vehicle (N=577) n (%)
Number of Ocular TEAEs	99	98
Number of Subjects with at Least One Ocular TEAE	77 (13.2%)	76 (13.2%)
Eye disorders	34 (5.8%)	40 (6.9%)
Visual acuity reduced [a]	16 (2.7%)	22 (3.8%)
Vitreous detachment	1 (0.2%)	0
Eye irritation	2 (0.3%)	3 (0.5%)
Conjunctival hemorrhage	2 (0.3%)	2 (0.3%)
Eye pain	1 (0.2%)	1 (0.2%)
Eye pruritus	2 (0.3%)	3 (0.5%)
Dry eye	1 (0.2%)	3 (0.5%)
Lacrimation increased	2 (0.3%)	0
Eyelid margin crusting	2 (0.3%)	2 (0.3%)
Vitreous floaters	2 (0.3%)	2 (0.3%)
Chalazion	0	1 (0.2%)
Conjunctival hyperemia	1 (0.2%)	0
Foreign body sensation in eyes	1 (0.2%)	2 (0.3%)
Ocular hyperemia	1 (0.2%)	0
Visual impairment	2 (0.3%)	0
General disorders and administration site conditions	47 (8.0%)	39 (6.8%)
Instillation site pain	46 (7.9%)	37 (6.4%)
Instillation site pruritus	1 (0.2%)	1 (0.2%)
Infections and infestations	2 (0.3%)	2 (0.3%)
Hordeolum	2 (0.3%)	1 (0.2%)
Investigations	1 (0.2%)	0

N=number of subjects in group, SAF=safety set, TEAE=Treatment-Emergent Adverse Event. Note: Subjects who participated in CYS-005 and who received vehicle in CYS-004 are counted in both the CyclASol 0.1% and Vehicle treatment arms. Percentages are based on the total number of subjects in each treatment group and integration pool.
a Preferred terms 'visual acuity reduced' and 'blurred vision' combined.
TEAEs are AEs that started or worsened on or after the date of first dose.
SOCs and PTs within a SOC are listed in order of descending frequency for All Subjects in the Pool B population. Subjects experiencing more than one TEAE within a given SOC or PT are counted once within that SOC or PT.
Source: ISS Table 14.3.1.4.1.

Reviewer's Comments: *In Studies CYS-003 and CYS-004, the most common adverse event reported was instillation site reactions 8%. Visual acuity reduced (3%) was the next most frequently reported adverse event.*

Laboratory Findings
Laboratory evaluations were not performed during Studies CYS-004 and CYS-005. CyclASol administered to the eye was previously established to have no systemic exposure. Ophthalmologic examinations of visual acuity, slit-lamp biomicroscopy, IOP, and dilated fundoscopy showed no meaningful differences between CyclASol and vehicle and no treatment-related changes.

8.3.4. Vital Signs

No analyses of vital signs and physical findings were performed. CyclASol was previously established to have no systemic exposure.

8.3.5. Electrocardiograms (ECGs)

ECGs were not performed in Studies CYS-004 and CYS-005.

8.3.6. Immunogenicity

Cyclosporine is not immunogenic. Immunogenicity was not assessed in the clinical studies.

8.3.7. Safety Analyses by Demographic Subgroups

Intrinsic factors were evaluated by analyzing subgroups by age, sex, race, ethnicity, iris color and baseline dryness score in the pooled ISS analyses. Overall, the incidence of ocular and non-ocular AEs, ophthalmologic examinations and the drop comfort analysis did not show any clear or consistent differences between the treatment groups in any of the subgroups.

8.4. Specific Safety Studies/Clinical Trials

There were no specific safety studies for this application.

8.4.1. Pediatrics and Assessment of Effects on Growth

This application does not trigger PREA. The applicant received an Agreed iPSP letter from the Agency dated February 20, 2020.

8.4.2. Overdose, Drug Abuse Potential, Withdrawal, and Rebound

CyclASol is not a narcotic and does not have abuse potential.

8.5. Safety in the Postmarket Setting

8.5.1. Safety Concerns Identified Through Postmarket Experience

CyclASol is not marketed in any country. Therefore, no post-marketing data have been produced.

8.5.2. Expectations on Safety in the Post-market Setting

No risk management activities are recommended beyond the routine monitoring and reporting of all adverse events.

8.5.3. Additional Safety Issues From Other Disciplines

There are no specific safety concerns from other disciplines.

8.6. Integrated Assessment of Safety

On December 6, 2022, the Applicant submitted a 120-day safety update to the NDA. The data lock for this safety update was October 31, 2022.

All Novaliq sponsored clinical studies were completed and reported at the time of the initial NDA submission and all safety data from these studies were included in the respective modules. Since the initial NDA there have been no new SAEs reported from Study SHR8028-301, which is conducted in China by Novaliq's partner Jiangsu Hengrui Pharmaceutical Co. for that region. As of the data lock date for this safety update, there are no ongoing clinical studies with cyclosporine ophthalmic solution and no new safety data for cyclosporine ophthalmic solution from clinical studies available.

Additionally, a Pubmed literature search was conducted after the data lock date. The search included the following search terms in the title or abstract of scientific publications for the product cyclosporine ophthalmic solution: dry eye, eye drops, cyclosporine or ciclosporin and for perfluorobutylpentane, semifluorinated alkane. In the period between initial NDA submission and data lock date for this safety update, the search revealed no hits for the product.

9. Advisory Committee Meeting and Other External Consultations

No Advisory Committee Meeting was held for this application. There were no new issues raised in the review of the application which were thought to benefit from an Advisory Committee Meeting.

10. Financial Disclosure - Covered Clinical Study (Name and/or Number): CYS-002, CYS-003 (ESSENCE-1) and CYS-004 (ESSENCE-2)

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: CYS-002:4 CYS-0003:9 CYS-004:27		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 0		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: Significant payments of other sorts: _____ Proprietary interest in the product tested held by investigator: _____ Significant equity interest held by investigator in Sponsor of covered study: _____		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☐	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☐	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) 0		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant) N/A

11. Labeling Recommendations

Prescription Drug Labeling
The Division of Medication Error Prevention and Analysis (DMEPA) finalized their review on 3/15/2023 with the following conclusions and recommendations:

> The proposed prescribing information (PI), container label, and carton labeling may be improved to promote the safe use of this product from a medication error perspective. We provide the identified medication error issues, our rationale for concern, and our proposed recommendations to minimize the risk for medication error in Section 4 for the Division and in Section 5 for Novaliq GmbH.

Table 2. Identified Issues and Recommendations for Division of Ophthalmology (DO)				
	IDENTIFIED ISSUE	RATIONALE FOR CONCERN	RECOMMENDATION	DO Response
Full Prescribing Information – Section 3 Dosage Forms and Strengths				
1.	As currently presented, the appropriate information to facilitate identification of the dosage form is not included.	A description of identifying characteristic can be used to help identify the product and is required by 21 CFR 201.57(c)(4)(ii).	Include the description of identifying characteristics of the dosage form, such as color (e.g., colorless to slightly yellow) and clarity (e.g., opalescent) in accordance with 21 CFR 201.57(c)(4)(ii). Similar to what is stated in *Section 11 Description*, "(b) (4) (b) (4)."	Agree, although the color and clarity in this case are not identifying characteristics.
Full Prescribing Information – Section 16 How Supplied/Storage and Handling				
1.	As currently presented, the appropriate information to facilitate identification of the dosage form is not included.	A description of identifying characteristic can be used to help identify the product and is required by 21 CFR 201.57(c)(iii).	Include the description of identifying characteristics of the dosage form, such as color (e.g., colorless to slightly yellow) and clarity (e.g., opalescent) in accordance with 21 CFR 201.57(c)(iii). Similar to what is stated in *Section 11 Description*, "(b) (4) (b) (4)."	Agree, although the color and clarity in this case are not identifying characteristics.

Table 3. Identified Issues and Recommendations for Novaliq GmbH (entire table to be conveyed to Applicant)				
	IDENTIFIED ISSUE	RATIONALE FOR CONCERN	RECOMMENDATION	DO Response
Prescribing Information – General Issues				
1.	As currently presented, the established name Cyclosporine is not presented in Tallman lettering format.	The name Cyclosporine is on the Office of Generic Drug's list of names for which the Tallman lettering format presentation is recommended.	Consider revising the established name "Cyclosporine" to read "cycloSPORINE," with the Tallman lettering format presentation. Apply this revision to the container label and carton labeling.	Disagree. Tallman letters are more crowded and more difficult to read for individuals with visual impairment.
Container Label(s)				

1.	As currently presented, the usual dosage statement includes "package insert".	The term "package insert" is inconsistent with terminology used in the Prescribing Information.	Revise the usual dosage statement to "Dosage: See Prescribing Information."	Agree.
Carton Labeling				
1.	[redacted] (b) (4)			
2.	As currently presented, we note that the product identifier is not included on the carton labeling.	The Drug supply Chain Security Act (DSCSA) requires, for certain prescription products, that the smallest saleable unit display a human-readable and machine-readable (2D data matrix barcode) product identifier. The DSCSA guidance on product identifier recommends a machine- readable (2D data matrix barcode) product identifier and a human-readable product identifier. Include the human-readable product identifier to the container label. The guidance also recommends the format of the human- readable portion be located near the 2D data matrix barcode as the following: NDC: [insert NDC] Serial: [insert serial number] LOT: [insert lot number] EXP: [insert expiration date]	We recommend that you review the guidance to determine if the product identifier requirements apply to your product's labeling. See *Guidance for Industry: Product Identifiers under the Drug Supply Chain Security Act – Questions and Answers* (July 2021). If you determine that the product identifier requirements apply to your product's labeling, add a placeholder for the product identifier on the carton labeling. Additionally, we recommend you ensure there is sufficient white space between the linear barcode and 2-D matrix barcode to allow barcode scanners the ability to correctly read each barcode.	The applicant should be compliant with the DSCSA requirements.

See Appendix of this review for final agreed labeling submitted May 30, 2023. The applicant has decided that that the separate "Instructions for Use" is not needed for the product, and it has been deleted from the labeling.

12. Post-marketing Requirements

While clinical signs of corneal endothelial cell dysfunction or death have not been observed with the use of cyclosporine ophthalmic solution, the health of prolonged exposure has not been fully investigated. Therefore, the Review Team considers that the investigations required under section 505(b) of the Federal Food, Drug, and Cosmetic Act do not include adequate tests by all methods reasonably applicable to show whether or not the drug is safe for use under the conditions prescribed, recommended, or suggested in its proposed labeling. A concurrently controlled, randomized, 12-month clinical study in which cyclosporine ophthalmic solution is dosed and corneal endothelial cell counts are compared to a concurrent control without cyclosporine ophthalmic solution is being required to be conducted and submitted.

PMR # 4454-1.

PMR Description
Conduct a randomized, controlled trial to evaluate the corneal endothelial health of eyes treated with cyclosporine ophthalmic solution, 0.1% by monitoring the number/density of corneal endothelial cells using specular microscopy at baseline and over a period of at least one year in at least 100 patients receiving (b) (4) ophthalmic solution.

PMR Schedule Milestones
Draft Protocol Submission: 10/2023
Final Protocol Submission: 12/2023
Trial Completion: 9/2026
Final Report Submission: 12/2026

There are no additional recommended post marketing risk evaluation and management strategies (i.e., REMS) for this drug product. There are no additional proposed risk management actions except the usual post marketing collection and reporting of adverse experiences associated with the use of the drug product.

13. Regulatory Action

NDA 217469, Veyve (cyclosporine ophthalmic solution) 0.1 % will be approved for the treatment of the signs and symptoms of dry eye disease.

Appendix

Attached is the final agreed labeling submitted May 30, 2023.

7 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

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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/

RHEA A LLOYD
05/30/2023 08:37:05 AM

WILLIAM M BOYD
05/30/2023 08:44:07 AM

WILEY A CHAMBERS
05/30/2023 09:36:26 AM

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:	217469
Supporting document/s:	SDN001
Applicant's letter date:	8-8-2022
CDER stamp date:	8-8-2022
Product:	Vevye® (cyclosporine ophthalmic solution 0.1%)
Indication:	For the treatment of the signs and symptoms of dry eye disease.
Applicant:	Novaliq GMBH c/o Strategic Drug Development Services LLC
Review Division:	Division of Pharm/Tox for Rare Diseases, Pediatrics, Urology and Reproductive Medicine/ Specialty Medicine (DPT-RPURM/SM) supporting the Division of Ophthalmology (DO)
Reviewer:	Erin Ruhland, PhD
Supervisor/Team Leader:	Kimberly Hatfield, PhD (acting for Lori Kotch, PhD)
Division Director:	Mukesh Summan, PhD
Project Manager:	Crystal Bland

Disclaimer
Except as specifically identified, all data and information discussed below and necessary for approval of NDA 217469 are owned by Novaliq GMBH or are data for which Novaliq GMBH has obtained a written right of reference.
Any information or data necessary for approval of NDA 217469 that Novaliq GMBH 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 NDA 217469.

TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

The Applicant has submitted a 505(b)(2) NDA application for Cyclosporin (CsA) Ophthalmic Solution, 0.1% for the treatment of dry eye disease. The new formulation contains a novel excipient, perfluorobutylpentane (F4H5). The active ingredient, CsA, has been previously approved in other suspension or solution formulations for the same indication and route of administration.

1.2 Brief Discussion of Nonclinical Findings

- The Applicant's relies on NDA 050573 (Sandimmune; Novartis Pharmaceutical Corp) for nonclinical support of systemic safety of CsA including genotoxicity, developmental and reproductive toxicity, and carcinogenicity. The bridge is established based on the large dose margin of the Listed Drug administered by intravenous injection and the Applicant's formulation, a topical ophthalmic drop.
- The Applicant's formulation is a solution primarily composed of the novel excipient perfluorobutylpentane (F4H5). The Applicant conducted systemic and ocular toxicology studies of F4H5 to qualify its use in the clinical formulations. At clinically relevant doses, no adverse toxicity was associated with F4H5. F4H5 was also negative for genotoxicity across *in vitro* and *in vivo* test systems.
- The Sponsor conducted a 6-month ocular toxicity study of the clinical formulation in rabbits. No adverse toxicity was associated with the vehicle or test article at doses which exceed the proposed clinical dose by 4.5-fold.

1.3 Recommendations

1.3.1 Approvability

- The NDA is approvable from a nonclinical pharmacology/toxicology perspective.

1.3.3 Labeling

The labeling portion of the nonclinical review will be contained within a separate review to be filed following agreement with the Applicant at the time of (potential) approval.

2 Drug Information

2.1 Drug

CAS Registry Number: 59865-13-3

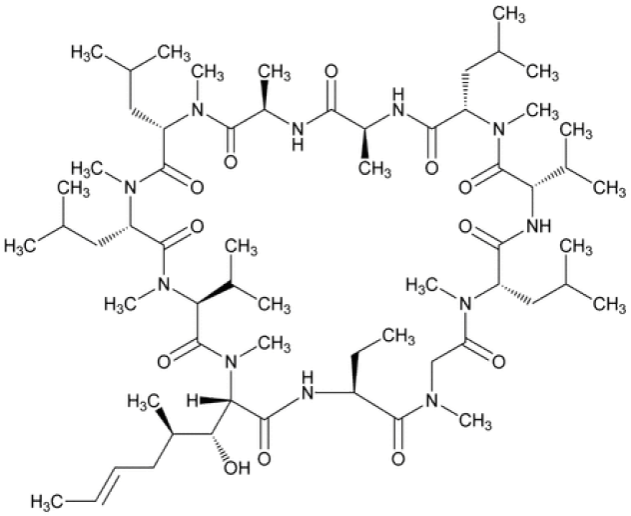
Generic Name: Cyclosporine ophthalmic solution, 0.1%, CyclASol (code name during product development)

Proprietary Name: Vevye®

Chemical Name: Cyclo[[(E)-(2S,3R,4R)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-2-aminobutyryl-N-methylglycyl-N-methyl-L-leucyl-L-valyl-N-methyl-L-leucyl-L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl-L-valyl]

Molecular Formula/Molecular Weight: $C_{62}H_{111}N_{11}O_{12}$ / 1202.6 g/mol

Structure:



Pharmacologic Class: Calcineurin inhibitor immunosuppressant (EPC)

2.2 Relevant INDs, NDAs, BLAs and DMFs

- IND 128,163
- DMF (b) (4)
- NDA 050573: Sandimmune (Cyclosporine A injection / oral capsules/solution); approved 11-14-1983

2.3 Drug Formulation

Qualitative and quantitative composition of cyclosporine ophthalmic solution

Component and Quality Standard	Function	Strength (Label Claim) 0.1% Quantity per Unit [mg/ 2mL]	Quantity per mL [mg]
Active ingredient			
Cyclosporine (USP)	API	2.00	1.00
Excipients			
Perfluorobutylpentane (Inhouse)			
Ethanol, (b) (4) (USP)			

(b) (4)

Density (20°C) of solution: 1.29 g/cm^3

2.4 Comments on Novel Excipients

Studies described in this section are reviewed later in the document.

The formulation includes a novel excipient, perfluorobutylpentance (F4H5), which was qualified for use in the clinical formulation for topical ocular administration based on the lack of ocular and systemic effects in the 6-month topical ocular toxicity studies in rabbits. Treatment with CyclASol vehicle (F4H5/(b) (4)% ethanol) at (b) (4) mg/eye/day F4H5 and F4H5 alone at (b) (4) mg/eye/day resulted in ocular dose safety margins that were 5.9- and 30-times higher, respectively, than the daily human ocular dose of F4H5 in the vehicle formulation.

Additionally, the Applicant performed systemic toxicity and genotoxicity studies of F4H5. Non-adverse, minimal to mild kidney and liver clinical pathology findings and organ weight increases were observed in the 6-month oral and topical ocular toxicity studies at higher doses of F4H5 in rats and rabbits, respectively, without any correlative histopathological findings. Systemic exposure (C_{max}) in these studies was 2.4-, 26- and 113- times higher, respectively, than the maximal F4H5 blood concentration at the daily human ocular dose of (b) (4) mg/day F4H5 in CyclASol vehicle. These findings were not observed in the 6-month topical ocular study with CyclASol vehicle at more clinically relevant doses of F4H5, and were associated with systemic exposure that was at least 10-times higher than that observed with a lower F4H5 dose with CyclASol vehicle in rabbits.

2.6 Proposed Clinical Population and Dosing Regimen

Vevye® (cyclosporine ophthalmic solution 0.1%) is indicated for the treatment of the signs and symptoms of dry eye disease. The dosing regimen is to instill one drop (10 µL/drop) in each eye twice daily. The daily human ocular dose of cyclosporine A in 0.1% Vevye® BID is 20 µg/eye (total bilateral dose 40 µg/day; 0.67 µg/kg/day based on 60 kg body weight).

Note: The total daily human ocular dose of the novel excipient, F4H5, is (b) (4) mg/eye/day.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacology

- Novaliq#13: Evaluation of the effects of different concentrations of CyclASol (Cyclosporine A in F4H5) and the vehicle F4H5 in a murine scopolamine model of experimental dry eye
- 15-130-0021: Evaluation of the effects of CyclASol in a murine scopolamine/CAE model of experimental dry eye

Safety Pharmacology

- 02G12019: 28 day toxicity study with oral application of F4H5 in Wistar Han rats
- 20-3-0106-12: 28 day toxicity study with F4H5 in New Zealand White rabbits after repeated administration into the conjunctival sac

Pharmacokinetics

- 495251: Single dose pharmacokinetics of F4H5 and cyclosporin A after administration in the eye in male and female New Zealand White rabbits
- N38D15816: Ocular pharmacokinetic study of CyclASol eyedrop formulation following a single instillation in pigmented rabbits
- 8345394: Pharmacokinetics, absorption, distribution, excretion of 14C-F4H5 following topical ocular administration to rabbits
- 8345395: Pharmacokinetics and distribution of ^{14}C-F4H5 following topical ocular administration to rabbits
- 03G12032: Toxicokinetics of radiolabelled [^{3}H]-F4H5 in rats
- 03N13501: In vitro metabolism of F4H5

Toxicology

- 20150063: Combined 3-month/6-month ocular toxicity study in New Zealand White rabbits with CyclASol ophthalmic solution including a 28-day recovery period and toxicokinetics
- 20-3-0116-12: Local tolerance study of cyclosporine A in F4H5 / ethanol in New Zealand White rabbits after repeated administration into the conjunctival sac for 28 days (including pharmacokinetic investigation)
- 02G12019: 28 day toxicity study with oral application of F4H5 in Wistar Han rats
- 02G12028: 26 week toxicity study with oral application of F4H5 in Wistar Han rats
- 20-3-0106-12: 28 day toxicity study with F4H5 in New Zealand White rabbits after repeated administration into the conjunctival sac

- 20-3-0120-12: 6 months toxicity study with F4H5 in New Zealand White rabbits after repeated administration into the conjunctival sac
- N38D17113: Compounds F4H5 and F6H8-evaluation of tear film changes following multiple daily instillation in albino rabbits
- 062188: Test for local effects after implantation (90 days period) with perfluorobutylpentane
- 053479: Test for delayed-type hypersensitivity (guinea pig maximization test) with perfluorobutylpentane
- 053478: Acute dermal irritation / corrosion with perfluorobutylpentane

Genotoxicity

- 16G10022: Bacterial reverse mutation test with F4H5
- 17G10021: In vitro mammalian chromosome aberration test (human lymphocytes) with F4H5
- 17G11002: Mammalian Erythrocyte Micronucleus Test with F4H5

3.2 Studies Not Reviewed

- 495255: Validation of analytical procedures for the analysis of F4H5 in EDTA rabbit blood by GC and mass spectrometric detection
- 15N12505: Validation of a headspace GC/MS method for the determination of F4H5 in rat and rabbit blood
- 02N12502: Comparative kinetics of F4H5 in blood following subcutaneous (s.c.), oral (p.o.) or intraperitoneal (i.p.) application in Wistar Han rats
- 15V12201: Validation of a HPLC/MS method for the determination of cyclosporin A (CsA) in rabbit blood, lacrimal gland, cornea, and conjunctiva
- I02F09014r1: Method validation RRLC-MS/MS method for determining cyclosporine A content
- MV-2015-1012: Method validation report for the determination of cyclosporine A in rabbit whole blood (lithium heparin) using LC-MS/MS
- N38D00214: Cyclosporin A validation of an RRLC-MS/MS method in pigmented rabbit sclera, lacrimal gland and retina
- 497004: 7 day MTD study with perfluorobutylpentane by ocular administration in New Zealand White rabbits
- 497002: Range finding and MTD study with perfluorobutylpentane by i.p. or s.c. dosing in Wistar rats
- 061709: In vitro cytotoxicity assay-evaluation of materials
- 190289: In vitro cytotoxicity assay: evaluation of materials for medical devices by extraction method and XTT dye with perfluorobutylpentane

3.3 Previous Reviews Referenced

Many of the nonclinical studies for the toxicology of perfluorobutylpentane (F4H5) were reviewed during clinical development under IND 128,163. Below, excerpts of the review

of these studies conducted by Dr. Ilona Bebenek and Dr. Mary Lewis are noted (DARRTs date: 12-2-2015).

4 Pharmacology

4.1 Primary Pharmacology

Novaliq#13: Evaluation of the effects of different concentrations of CyclASol (Cyclosporine A in F4H5) and the vehicle F4H5 in a murine scopolamine model of experimental dry eye

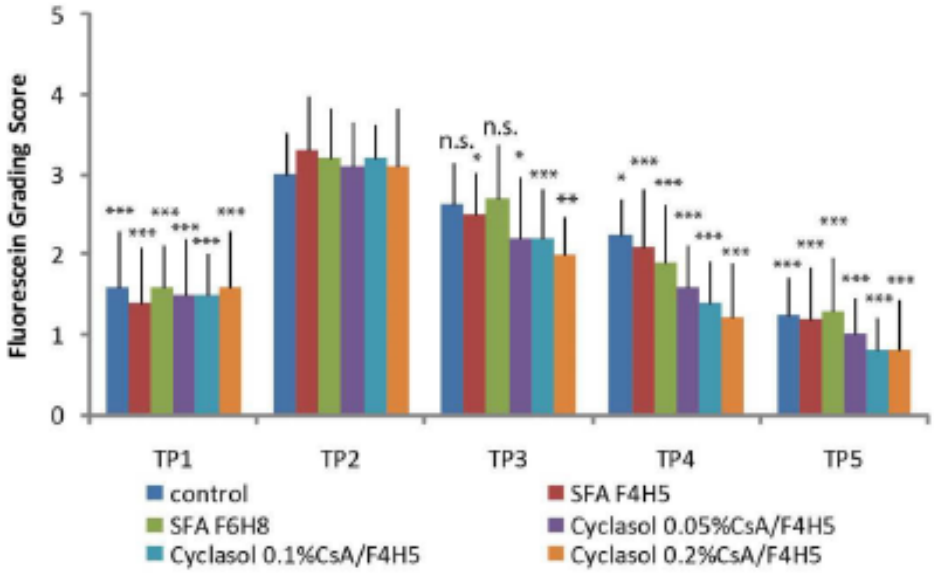
- This study determined the activity of the Applicant's CsA formulation in a model of experimental dry eye in C57BL/6 mice.
- On Day 1, all groups received scopolamine administration (0.1 mg/day; Days 1 to 14) by subcutaneous implanted osmotic pumps. The animals were placed in desiccating controlled environment chambers (low humidity, high-airflow) during this time. Animals were housed in normal controlled housing on Days 15 – 35.
- The CsA formulations or the vehicle controls were administered three times daily on Days 11 through 35. Treatment groups:

Group No.	No. of An.	Baseline Exam	Dry Eye Induction	Treatment (Dose Level)	Dosing Regimen/ROA	Terminal Time Point
1	5	Production of tear fluid / Fluorescein Staining Day 0	Scopolamine 0.1 mg/ animal/day on Days 0 - 14 via SC implanted osmotic pumps	Untreated Control	NA	Day 35
2	5			Vehicle (F4H5)	5 µL to each 3x daily by topical application on Days 11 – 35	
3	5			Vehicle (F6H8)		
4	5			0.05 % CyclASol (Cyclosporine A in F4H5)		
5	5			0.1 % CyclASol (Cyclosporine A in F4H5)		
6	5			0.2 % CyclASol (Cyclosporine A in F4H5)		

NA = not applicable

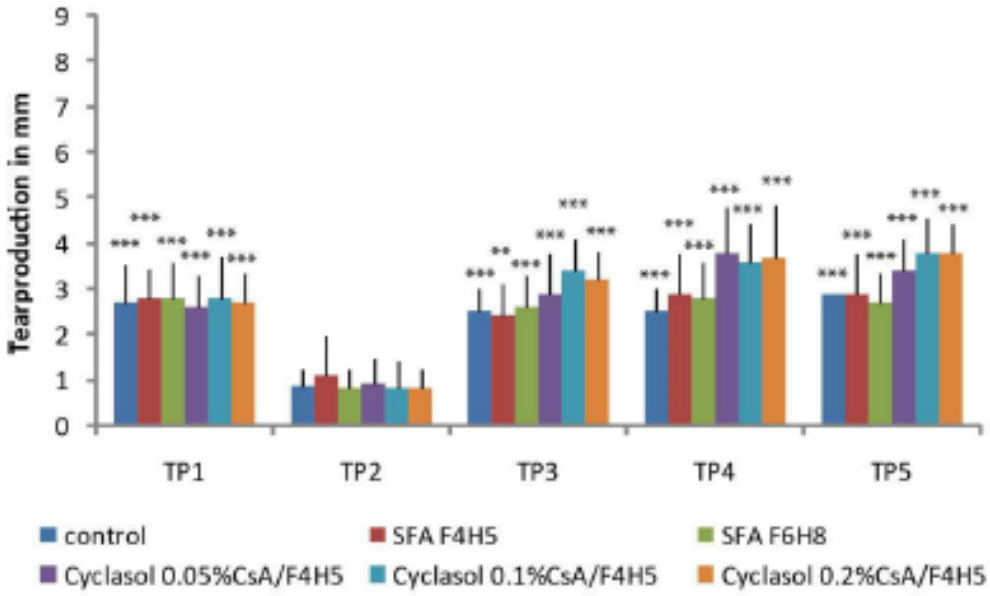
- Animals were evaluated on days 14, 21, 28, 35 by fluorescein staining prior to the first topical dose of the day.
- All groups demonstrated a significant increase of epithelial damage as shown by corneal fluorescein staining in mice on Day 14 [Time Point 2 (TP2)] after environmental dry eye treatment (EDE) compared to baseline (Day 0; TP1).

- Treatment with 0.05 %, 0.1 %, 0.2 % CyclASol as well as F4H5 treatment significantly reduced fluorescein staining after end of the desiccating stress on Day 21 (TP3), whereas untreated control and F6H8 group continued increased fluorescein levels at TP3. On Day 28 (TP4) fluorescein grade was decreased in all groups compared to TP2.



Data are the mean SD for n=10 eyes per group. The figure above shows absolute mean grading per group.P-values ≤ 0.05 were considered to be significant (* p≤0.05, ** p≤0.001, *** p≤0.0001). Significances refer to TP2.

- Tear production was reduced in all groups at the end of desiccating stress (TP2) independent of the treatment regimen, but significantly improved at TP3 (Day 21). Comparing the different groups after treatment, tear production was significantly increased in the 0.1 % CyclASol group compared to the vehicle groups and untreated control at TP3. At TP4 and TP5, 0.05 %, 0.1 % and 0.2 % CyclASol treatment led to an increased tear production compared to vehicle and untreated groups.



15-130-0021: Evaluation of the effects of CyclASol in a murine scopolamine/CAE model of experimental dry eye

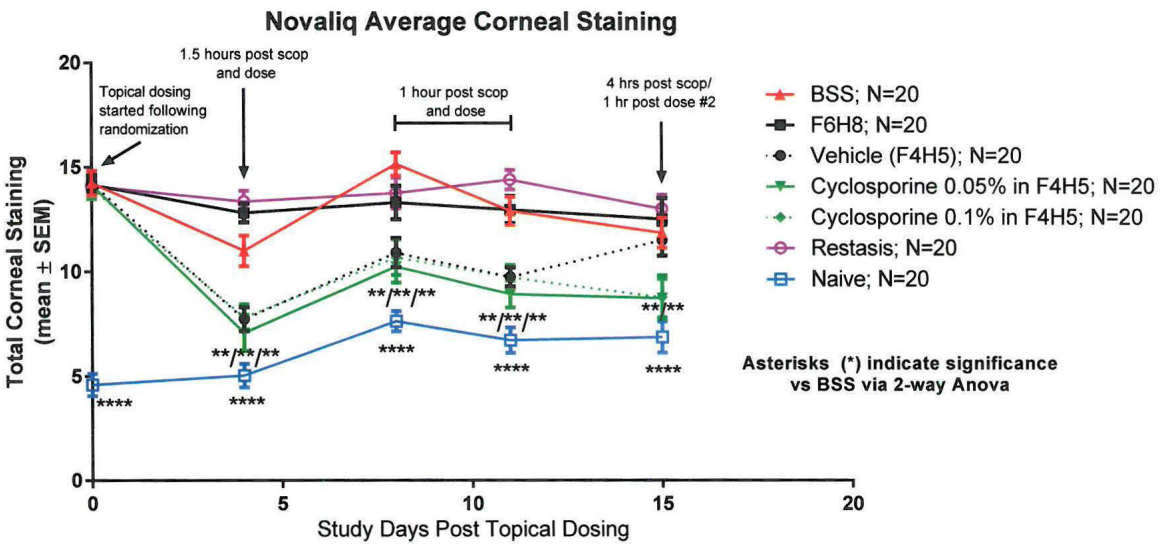
- In the dry eye model described above, the Applicant determined the activity of the CsA formulations with treatment commencing at the time of desiccating treatment.
- On Day -3, all groups received scopolamine administration (0.1 mg/day; Days -3 to 15) by subcutaneous injection. The animals were placed in desiccating controlled environment chambers (low humidity, high-airflow) during this time.
- The CsA formulations or the vehicle controls were administered three times daily on Days 0 through 16. Treatment groups:

Group No.	No. of An.	Baseline Fluorescein Exam [a]	Dry Eye Induction	Treatment (Dose Level)	Dosing Regimen/ROA	Terminal Time Point
1	10	Day -3	Scopolamine 0.375 mg/ animal 2x daily on Days 0 - 15 via SC injection into back	Vehicle 1 (F4H5)	3 µL to each 3x daily by topical application on Days -3 – 16	Day 16
2	10			Vehicle 2 (F6H8)		
3	10			BSS		
4	10			CyclASol – 0.05%		
5	10			CyclASol – 0.1%		
6	10			Restasis		
7	10		NA	Naive	NA	

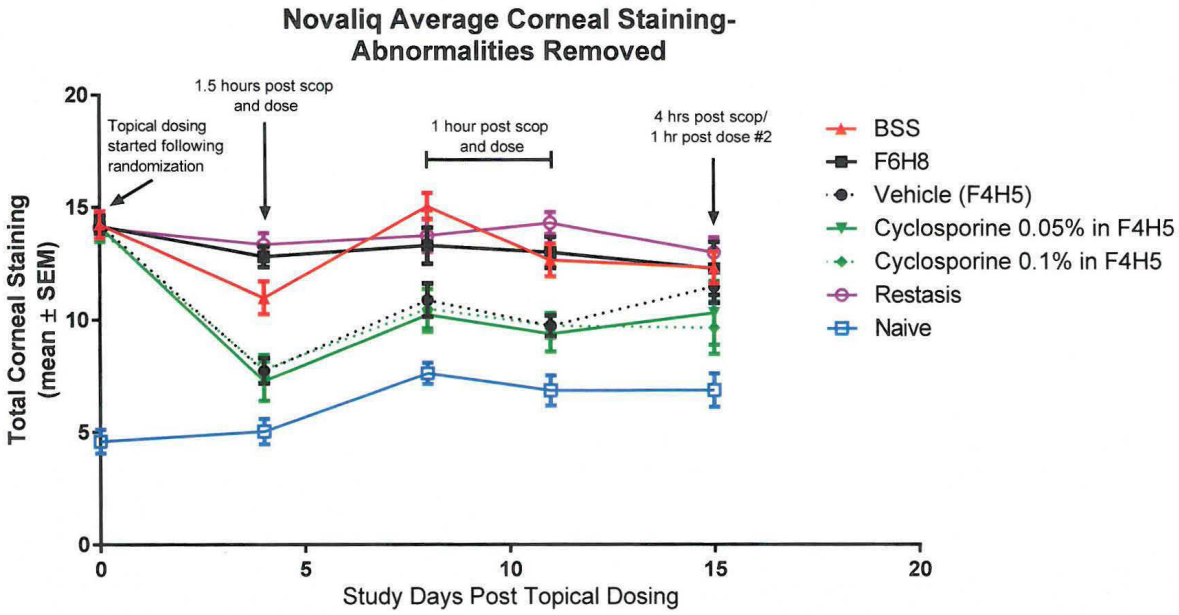
NA = not applicable

[a] N = 30 spares will be utilized to ensure enrollment of mice with the lowest baseline eye evaluations in Groups 1 - 7.

- Animals were evaluated by corneal fluorescein staining on days 4, 8, and 11 at 1 hour post morning treatment, on Day 15 at 1 hour post 2nd dose, and on Day 16.
- The results indicated that the CsA formulations had an effect on decreasing corneal staining. However, the vehicle F4H5 also showed effects in comparison to the remaining controls (balanced salt solution [BSS], F6H8 and Restasis). All groups formulated in this vehicle had statistically lower corneal staining compared to BSS and F6H8 when evaluating the animals approximately one hour post scopolamine and topical dosing. When the evaluations were pushed to four hours post scopolamine (Day 15), the beneficial effect of the vehicle compared to the active formulations was diminished. The Applicant reanalyzed the raw data by removing all the animals with abnormalities such as corneal scratches or those that had a reduction in scopolamine dose on the day of evaluation (due to body weight loss). After performing this reanalysis, the data on Day 15 shows no separation between vehicle (F4H5) and the active CsA/F4H5 formulations (second figure below).



Data are the mean ± SEM for n=20 eyes per group. Data were analyzed using a 2-way ANOVA compared to BSS, Vehicle (F4H5), and F6H8. The figure above shows significance compared to BSS only. The following tables show the significance between each group and the comparator on each study day.



4.3 Safety Pharmacology

Cyclosporine A

Safety pharmacology of CsA was not evaluated due to a lack of systemic exposure in human and animal studies after ocular administration.

F4H5: Novel Excipient
Safety pharmacology parameters of F4H5 were evaluated in pivotal 28-day toxicology studies (cardiovascular-rabbits; CNS and respiratory- rats) and are also presented in section 6 of this review under each respective study.

In rabbits administered 513, 1026 or 1538 mg/eye F4H5 per day for 28 days via subconjunctival administration, heart rate was significantly higher in females dosed with 513 mg/day (+20%; p≤ 0.05) or 1026 mg/day (+18%; p≤ 0.05) on Day 28. Heart rate of females dosed with 1538 mg/day was also higher than controls (+10%) but this difference was not statistically significant. This effect was not considered adverse since increase in heart rate was relatively small and did not increase with dose.

In rats dosed with 500, 1000 or 2000 mg/kg/day via oral gavage, number of rearings was significantly increased during one 15 min interval in females dosed with 500 (48%) and 1000 mg/kg (+56%) on Day 28. Only the 1000 mg/kg value reached statistical significance (p<0.05) and the effect was not observed in the highest dose nor in male animals. Also, time in rest was significantly increased (+9%) and time in movement significantly decreased (-46%) in males dosed with 1000 mg/kg, as measured in one interval. The effect was not observed in females. Due to the inconsistency of these effects, lack of dose response, and presence in one sex, the results did not show a treatment-related effect. No test article-related effects were observed in functional observational battery tests (performed on week 1 and week 4 of 28 day study).
A decrease in inspiratory time at 15-20 min after treatment with the 1000 mg/kg (31%) and 2000 mg/kg (26%) dose of F4H5 in rats, was the only statistically significant difference in respiratory parameters, compared to the control group on Day 0 (males and females combined). These effects were not considered adverse since they were transient (no changes up to 180 min post dose; Day 0 only) and small in value.

5 Pharmacokinetics/ADME/Toxicokinetics

Reviewer's note: In the clinical studies conducted by the Applicant, only single F4H5 concentrations above the lower limit of quantification (i.e. (b) (4) ng/mL) were observed at single time points in some of the subjects (maximum observed concentration was (b) (4) ng/mL in CYS-001 and (b) (4) ng/mL in CYS-002).

5.1 PK/ADME

Study #495251; GLP: Single Dose Pharmacokinetics of F4H5 and Cyclosporine A after Administration in the Eye in Male and Female New Zealand White Rabbits

Twenty-four male and female New Zealand White rabbits were treated with a single topical ophthalmic dose of 200 µL of 0.5 mg/mL Cyclosporine (0.05%) in F4H5/ethanol ((b) (4)) into each eye (into the subconjunctival sac) for a total of 0.2 mg Cyclosporine A and (b) (4) mg F4H5 per rabbit. The bioavailability of the vehicle F4H5 and of the active substance CsA after administration into the eye were examined. A control group

consisting of 6 animals (3 males and 3 females) was not dosed. Blood and eyes were collected from three animals per sex per time point at 0.133, 0.25, 0.5, 1, 2, 4, 8 and 24 hours after dosing. Subsequently, different layers of the eyes were dissected, i.e. conjunctiva, cornea, sclera, aqueous humor and lacrimal gland for determination of F4H5 exposure. Cyclosporine A was measured in the cornea and the lacrimal gland using a HPLC method with tandem mass spectrometric detection.

F4H5 in blood

After administration into the eye, the concentration of F4H5 was measured in blood. The blood concentration increased rapidly and C_{max} was reached at the first blood sampling time at 8 minutes (0.133 h) after dosing in males and females (Table 1). The last time point with measurable concentrations was 4 hours after dosing. Exposure in males and females was comparable, with an AUC_{last} of 48.7 and 32.2 hr*ng/mL and a C_{max} of 74.0 and 45.1 ng/mL for males and females respectively. F4H5 was rapidly eliminated from the body with an apparent terminal half-life (t½) of 0.931 hour in males.

Table 1: Pharmacokinetic parameters of F4H5 in blood after administration to the subconjunctival sac

Gender	Male	Female
Dose amount per eye (µL)	200	200
Dose Level F4H5 (mg per rabbit)		(b) (4)
t_{last} (hr)	4.0	4.0
t_{max} (hr)	0.133	0.133
C_{last} (ng/mL)	1.38	2.13
C_{max} (ng/mL)	74.0	45.1
#C_{max} (ng/mL/mg)	0.145	0.0885
AUC_{last} (hr*ng/mL)	48.7	32.2
#AUC_{last} (hr*ng/mL/mg)	0.0956	0.0632
AUC_{∞} (hr*ng/mL)	50.5	37.6*
#AUC_{∞} (hr*ng/mL/mg)	0.0991	0.0738*

* : approximation; # : dose normalized to 1 mg

Excerpted from the Sponsor's study report, page 7

F4H5 in ocular tissues

The tissue concentration of F4H5 increased rapidly with a t_{max} between 0.133 and 0.5 hours after dosing; in the conjunctiva of females a t_{max} of 8 hours was reported (Table 2). For all tissues, t_{last} was 24 hours. The exposure to F4H5 in tissues was higher in females, than males, ranging from 2-21-fold depending on the tissue; the reason for this gender difference is unclear. For both genders the highest exposures and C_{max} values were reported in the conjunctiva (which was the dosing site), followed by the cornea, sclera and aqueous humor.

Table 2: Pharmacokinetic parameters of F4H5 in eyes after administration to the subconjunctival sac

Tissue	Aq humour		Conjunctiva		Right Cornea		Sclera	
Gender	Male	Female	Male	Female	Male	Female	Male	Female
Dose amount per eye (µL)	200	200	200	200	200	200	200	200
Dose Level F4H5 (mg per rabbit)	(b) (4)							
t_{last} (hr)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
t_{max} (hr)	0.133	0.25	0.50	8.0	0.133	0.133	0.25	0.133
C_{last} (ng/g)	2.54	3.87	62.7	206	21.3	47.6	6.93	23.1
C_{max} (ng/g)	7.79	14.3	2480	6140	365	803	121	275
#C_{max} (ng/g/mg)	0.0153	0.0281	4.87	12.1	0.717	1.58	0.238	0.540
AUC_{last} (hr*ng/g)	53.7	115	3100	66400	739	1370	186	696
#AUC_{last} (hr*ng/g/mg)	0.105	0.226	6.09	130	1.45	2.69	0.365	1.37

: dose normalized to 1 mg

Excerpted from the Sponsor's study report, page 7

Cyclosporine A in ocular tissues

Cyclosporine A was measured in the cornea and the lacrimal gland. The tissue concentration increased rapidly with tmax between 0.133 and 0.25 hours after dosing (Table 3). Tmax was less than one hour, with the exception of the male corneas, where it was 4 hours. For all tissues tlast was 24 hours. No sex differences for Cmax and AUC were observed. In the cornea a much higher concentration of Cyclosporine A was measured than in the lacrimal gland. Where accurate determination was possible (lacrimal gland males), the apparent terminal half-life (t½) was 4.56 hours.

Table 3: Pharmacokinetic parameters of Cyclosporine in eyes after administration to the subconjunctival sac

Tissue	Left Cornea		Lacrimal Gland	
Gender	Male	Female	Male	Female
Dose amount per eye (µL)	200	200	200	200
Dose Level Cyclosporin A (mg per rabbit)	0.20	0.20	0.20	0.20
t_{last} (hr)	24.0	24.0	24.0	24.0
t_{max} (hr)	4.00	0.133	0.133	0.250
C_{last} (ng/g)	470	504	4.47	44.0
C_{max} (ng/g)	1140	1320	94.4	127
#C_{max} (ng/g/mg)	5700	6600	472	636
AUC_{last} (hr*ng/g)	17400	16800	1210	1080
#AUC_{last} (hr*ng/g/mg)	87000	84000	6060	5380
AUC_{∞} (hr*ng/g)	28900*	32600*	1240	n/c
#AUC_{∞} (hr*ng/g/mg)	144500*	163000*	6210	n/c

* : approximation; # : dose normalized to 1 mg; n/c : could not be calculated

Excerpted from the Sponsor's study report, page 7

Study N38D15816: Ocular pharmacokinetic study of CyclASol eyedrop formulation following a single instillation in pigmented rabbits

- This study determined the ocular absorption of cyclosporine A in the cornea, conjunctivae, aqueous humor, sclera, lacrimal gland, retina and whole blood after

a single instillation in both eyes of pigmented rabbits (n=3/time-point). Other formulations and a marketed formulation were also studied but these results are not presented in this review.

- Animals were treated with CsA formulations bilaterally; 0.011mL/drop.
- Following a single instillation of Cyclosporine A formulations in both eyes of rabbits, the highest exposure was found in the general order of cornea > conjunctiva > sclera > lacrimal gland > retina > whole blood. The maximum concentrations of Cyclosporine A were mainly observed 8-15 minutes post dosing, except for sclera which showed a T_{max} of 1 hours.

	Cmax (ng/g)	**Tmax (h)**	**$T_{½}$ (h)**	**$AUC_{0.133 - 24hr}$ (ng·hr/mL)**
Cornea	1051	0.133	12.4	7741
Conjunctiva	320	0.25	5.0	1313
Lacrimal gland	15.9	0.133	4.8	48.2
Retina	4.1	0.133	NA	0.2
Sclera	80.3	1	9.1	796
Blood	Not detected*	NA	NA	NA

***** The lower limit of quantitation (LLOQ) was 0.25 ng/mL for whole blood.

Study #8345394: Pharmacokinetics, absorption, distribution, excretion of ^{14}C-F4H5 following topical ocular administration to rabbits

- The pharmacokinetics, absorption, distribution, and excretion of ^{14}C-Perfluorobutylpentane (F4H5) was assessed after a single or multiple oral administrations to rats.
- Rats received either a single dose of ^{14}C-F4H5 or twice daily doses of ^{14}C-F4H5, approximately 12 hours apart for three days and on the fourth day received a single dose in the a.m. (a total of seven doses); the dose of F4H5 administered was 1000 mg/kg.
- Following a single administration to rats, low levels of radioactivity were detected in blood and plasma indicating that low levels of substance-related radioactivity was absorbed and entered systemic circulation. Radioactivity was first detected at 2 hours postdose. The maximum concentrations of radioactivity in blood and plasma were observed at 12 hours postdose; concentrations declined after this time and were still detectable at 48 hours postdose (the last collection time point).
- After multiple oral administrations, mean concentrations of ^{14}C-F4H5 in blood and plasma were higher than those observed following a single dose administration by at least 2.6-fold, indicating that some systemic absorption had occurred. The mean maximum concentrations in blood and plasma were observed at 4 and 0.5 hours postdose, respectively.

- The highest levels of substance-related radioactivity were found in GI associated tissues or contents. The highest concentrations were in the general order of large intestine contents > small intestinal contents > large intestine > small intestine > rectum > stomach > fat > pancreas > adrenal glands > stomach contents > liver > salivary glands > lymph nodes > pituitary gland > thyroid.
- The majority of the administered dose was eliminated in feces. Most of the radioactivity was excreted in the first 24 hours postdose, indicating rapid elimination of substance-related compound. Radioactivity in urine accounted for less than 0.5% of the dose administered. Radioactivity recovered in the air traps accounted for a mean of approximately 13% of the dose over the 0-168 hours postdose collection interval following a single dose.
- The high percentage recovery in feces and low recovery in urine and remaining in the carcass indicated that the majority of the administered test article was not absorbed.
- Low levels of radioactivity recovered in bile indicates that biliary excretion was not a route of elimination for substance-related compound.

Study #8345395: Pharmacokinetics and distribution of ^{14}C-F4H5 following topical ocular administration to rabbits

- The pharmacokinetics and distribution of ^{14}C- Perfluorobutylpentane (F4H5) were assessed after single or multiple topical ocular administration to rabbits.
- Animals received a single topical ocular administration of ^{14}C-F4H5 to one or both eyes. A separate group of animals received twice daily doses (approximately 8 hours apart ± 15 minutes) administered to both eyes for four consecutive days, and on the fifth day received a single dose in the a.m. to both eyes (a total of nine doses). The dose administered was 45 mg/eye/dose in a volume of 0.035 mL. Blood, selected ocular tissues (including tears collected prior to sacrifice), and selected systemic tissues were collected from all animals at sacrifice.
- Following single or multiple doses, very low levels of radioactivity were detected in blood and plasma at sporadic time points. While the values indicated that some substance-related compound may have entered the systemic circulation, the concentrations were very low, indicating that absorption was limited following topical ocular administration.
- Concentrations of substance-related compound were observed in the majority of the systemic tissues collected. However, concentrations were low, and the percentage of administered radioactivity recovered in systemic tissues was ≤0.2%, further demonstrating that limited systemic absorption occurred. The highest concentrations were observed in the general order: whole head > kidneys > small intestine > fat > liver > heart > stomach > brain > large intestine with cecum. As the whole head was analyzed without brain and eyes, the radioactivity detected in this tissue likely represented administered dose drained via the nasal passages.

- The highest concentrations of substance-related ^{14}C-F4H5 in ocular tissues following a single topical ocular administration to both eyes were observed in the general order: tears > palpebral conjunctiva > meibomian glands > bulbar conjunctiva > cornea > accessory lacrimal gland > anterior sclera, observed between 0.25 and 1 hour postdose. Concentrations of radioactivity were observed at 24 hours postdose in all anterior ocular tissues with the exception of the lacrimal glands.
- Concentrations of substance-related ^{14}C-F4H5 in pigmented tissues (iris-ciliary body and choroid-RPE) were BLQ, showing no association or retention of ^{14}C-F4H5 in melanin-containing tissues.
- Following multiple topical ocular administration, the distribution of substance-related radioactivity occurred mostly into the anterior section of the eye, with the highest concentrations observed between 0.25 and 2 hours postdose in the order: tears > meibomian glands > palpebral conjunctiva > cornea > bulbar conjunctiva > accessory lacrimal gland > anterior sclera, with little or no distribution to the posterior section.

Study #03G12032; GLP: Toxicokinetics of Radiolabeled ^{3}H-F4H5 in Rats

- Four male rats per group were dosed with 35 mg radioactive F4H5 (^{3}H-F4H5) as a single dose or daily for eight days via application to the subconjunctival sac; animals were sacrificed two hours after the last dose. F4H5 levels were evaluated in the eyes and blood of these animals. Four male rats and four female rats received a single dose of 1000 mg/kg radioactive F4H5 via oral gavage; the blood and tissues of these animals were evaluated for kinetics and tissue distribution of F4H5 at 24 hours (blood was collected at 2, 4, 8 and 24 hours). In addition, two male and two female rats received a single dose of 1000 mg/kg F4H5 via oral gavage; these animals were used for excretion and tissue distribution analysis seven days after the single dose.

- <u>Ocular administration</u>
  - Analysis of eyes and blood revealed only traces of radioactivity (0.001 % of the dose applied) in the eyes of animals sacrificed 2 hours after a single ocular administration of radioactive F4H5. Blood radioactivity (0.004 % of the dose applied) was detected in one out of four animals only. Trace amounts of radioactivity were recovered in the eyes (< 0.03% of the dose applied) and blood samples (< 0.09% of the dose applied) of rats dosed via daily subconjunctival sac administration for 8 days. The results suggest that F4H5 does not accumulate in the eye after repeated ocular application.

- <u>Oral administration</u>
  - During the first 24 hours after a single oral application of [^{3}H]-F4H5, blood radioactivity levels increased slightly, to levels of 0.15 to 0.38 % and 0.11 to 0.25 % of the dose applied in males and females, respectively. Fecal excretion of [^{3}H]-F4H5 accounted for 65 ± 4 % of the dose applied,

whereas only 1.7 ± 0.2 % and 0.09 ± 0.02 % were recovered in urine and cage wash, respectively. 11.6 ± 0.5 % of the ^{3}H-activity was detected in the exhaled air. The total activity recovered in the sum of organs and tissues (equivalent to residual carcass) after 24h (single oral administration; 24-hour collection time point) accounted for 1.9 to 4.8 % and 1.1 to 2.5 % in males and females, respectively. The highest levels of radioactivity were detected in muscle and adipose tissue (males and females; 24 hour time point).

- According to the Applicant, the observed increase in radioactivity in blood, organs and tissues, albeit of limited extent, was somewhat unexpected and not plausible, as F4H5 itself is non-polar. This raised the suspicion that [^{3}H]-F4H5 may be unstable and other radioactive breakdown products might contribute to the evenly distributed background radioactivity observed after oral administration. The results of a separate study, "Stability Testing of tritium-labelled F4H5" suggest that the radioactivity detected in blood, organs and tissues originated mostly from tritium labelled water. In view of this fact, the results of Study 03G12032 suggest a very low absorption of F4H5 after oral administration. The slight increase of blood radioactivity levels (oral administration groups) likely reflects the formation of tritium labelled water.

***In vitro* Metabolism of F4H5 (Study #03N13501; non-GLP)**

The metabolic stability of F4H5 was assessed in microsomal preparations of rat, rabbit and human S9 fractions. The tested concentrations ((b) (4) µg/mL as low dose, (b) (4) µg/mL as high dose) were selected based on blood concentrations observed in rats following oral or intraperitoneal administration. F4H5 at the respective concentration was incubated with liver microsomes of the different species for 1 hour each; control samples contained testosterone. After the incubation time, the remaining amount of F4H5 in the test samples was quantified by mass spectrometry. Testosterone and the known metabolite 6β-hydroxytestosterone in control samples were analyzed by high performance liquid chromatography. While the control samples readily metabolized testosterone as expected (indicating good metabolic performance of the test system), F4H5 was not metabolized at all. Recovery of intact F4H5 from the samples after incubation ranged from 96% to 120% (Table 4). The Applicant acknowledged recovery values higher than 100% but an explanation was not provided, with the exception that variability was preanalytical or analytical.

Unexpectedly, recoveries were lower in the heat-inactivated samples (75%-92%), which were intended as controls without metabolic capacity. As metabolism cannot account for the loss of F4H5 in these samples, other factors, such as increased protein binding, were discussed as possible causes for this finding. In conclusion, F4H5 was not metabolized by liver microsomes of rats, rabbits or humans.

Table 4: Recovery of F4H5 after microsomal incubation for 60 minutes

No.	Fraction	Species	Substrate Concentration	Incubation Time	F4H5 recovered (ug/mL)	F4H5 recoverd (%)	% SD
			(b) (4)		(b) (4)		
1	microsomes	human		60'		120	1.3
2	microsomes	human		60'		98	8.8
3	microsomes	rat		60'		112	9.2
4	microsomes	rat		60'		114	7.0
5	microsomes	rabbit		60'		108	8.3
6	microsomes	rabbit		60'		96	12.0
7	S9-fraction	human		60'		100	9.1
8	S9-fraction	human		60'		103	10.2
9	microsomes heat-inactivated	human		0'		88	2.1
10	microsomes heat-inactivated	human		0'		75	8.8
11	microsomes heat-inactivated	human		60'		92	8.7
12	microsomes heat-inactivated	human		60'		88	4.6

Excerpted from the Sponsor's study report, page 11

6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: Combined 3-month/6-month ocular toxicity study in New Zealand White rabbits with CyclASol ophthalmic solution including a 28-day recovery period and toxicokinetics

Study no.: 20150063

Study report location: \\CDSESUB1\EVSPROD\nda217469\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\20150063\20150063-pre-clinical-study-report-1.pdf

Conducting laboratory and location: (b) (4)

Date of study initiation: 2/11/2016

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: CyclASol 0.05%; Batch 150882
CyclASol 0.15%; Batch 150884

Key Study Findings

- No adverse toxicity was reported following ocular instillation of the high dose (0.15% CsA; TID) or clinical vehicle for 6-months in New Zealand White rabbits
- The NOAEL 0.18 mg/eye/day provides a safety margin of 4.5-fold compared to the proposed clinical dose of 0.04 mg/eye/day.

Methods

Doses:	CyclASol 0.05%: 0.06 mg/eye/day CyclASol 0.15%: 0.18 mg/eye/day
Frequency of dosing:	Three times daily (TID) for 3 or 6 months
Route of administration:	Bilateral topical ocular instillation
Dose volume:	0.04mL/drop
Formulation/Vehicle:	Clinical formulation (F4H5; saline control also included
Species/Strain:	Rabbit / New Zealand White
Number/Sex/Group:	3-month interim sacrifice: 3/sex/group Terminal sacrifice (6-month): 6/sex/group Recovery (1 month after terminal): 3/sex/group
Age:	17 – 21 weeks
Weight:	2.91 – 4.39 kg
Unique study design:	A marketed formulation of CsA was also included as a test group in the study; those results are omitted from this review.
Deviation from study protocol:	No deviations affected the conduct or interpretability of the study.

Observations and Results

Mortality

- Three (3) animals died prior to scheduled sacrifice. The deaths were not considered treatment related.
    - Animal 91 from the high-dose recovery group refused food, which was considered to be a reaction to stress. The animal was euthanized on study day 12 due to undernourishment. This animal was replaced by animal No. 103.
    - Animal 76 from the low-dose group died during blood sampling on study day 1. This was considered to be related to high stress of the animal during the procedure. This animal was exchanged by animal 104.
    - Animal 56 from the vehicle control was found dead on study day 89. This animal was not replaced.

Clinical Signs (cageside: twice daily; detailed examination: prior to study and once weekly)

- No changes in clinical signs attributed to the test article.

Body Weights (prior to study and weekly)

- No changes in body weight attributed to the test article.

Ophthalmoscopy (Fundoscopy: prior to study and prior to scheduled sacrifice; slit-lamp biomicroscopy: prior to study and Weeks 2, 3, 5, 14 and 27; modified Hackett-McDonald scoring)

- Findings considered to not be of toxicological importance (sporadic, resolving, or also occurring with equal incidence or severity in vehicle / saline treated eyes) included conjunctival congestion (Grade 1) and conjunctival discharge (Grade 1).

Hematology (prior to study and prior to scheduled sacrifice)

- No changes in hematological parameters attributed to the test article.

Clinical Chemistry (prior to study and prior to scheduled sacrifice)

- No changes in clinical chemistry parameters attributed to the test article.

Gross Pathology

- No changes in macroscopic observations attributed to the test article.

Organ Weights

- No changes in organ weights attributed to the test article.

Histopathology

Adequate Battery: Yes; standard battery of systemic tissues and eyes with optic nerve and Harderian gland, including eye lids with tarsal glands and lacrimal glands.

Peer Review: Yes

Histological Findings

- At the 3-month sacrifice, hepatocellular hypertrophy (centrilobular) was recorded in two low dose and in one high dose CyclASol® -treated females. No corollary findings were noted and a relationship to the test article was not concluded.

Toxicokinetics

Arithmetic mean (range) toxicokinetic parameters of cyclosporine A in male and female New Zealand White rabbits after repeated ocular administrations of clinical CsA formulation

Day	Gender	C_{max} (ng/mL)	t_{max}[a] (h)	C_{last} (ng/mL)	t_{last}[a] (h)	AUC_{0-t} (h*ng/mL)	AUC_{0-4} (h*ng/mL)	AUC_{4-8} (h*ng/mL)	AUC_{8-24} (h*ng/mL)	RA C_{max}	RA AUC_{0-t}
Group 3 (3 x daily 40 µL of 0.05% CyclASol® per eye = 120 µg/day cyclosporine A)											
1	f	0.234 (0.105-0.338)	8.17 (1.00-8.17)	0.180 (0.105-0.261)	24.00 (8.17-24.00)	3.86 (0.429-6.04)	0.470 (0.103-0.824)	0.606 (0.309-0.793)	3.47 (2.75-4.76)	n.a.	n.a.
92	f	0.583 (0.145-2.30)	8.08 (4.17-24.00)	0.202 (0.145-0.315)	16.08 (8.17-24.00)	4.31 (0.897-10.5)	0.740 (0.389-0.957)	1.53 (0.486-5.16)	4.05 (2.98-4.71)	1.16 (0.68-2.11)	1.34 (0.26-3.78)
183	f	0.244 (0.146-0.334)	8.17 (4.17-8.17)	0.132 (0.109-0.177)	24.00 (24.00-24.00)	4.06 (3.16-5.09)	0.571 (0.242-0.775)	0.723 (0.540-1.00)	2.77 (2.12-3.46)	1.14 (0.69-1.94)	2.23 (0.80-7.65)
1	m	0.620 (0.254-1.50)	8.08 (0.00-8.17)	0.141 (0.107-0.190)	24.00 (8.17-24.00)	4.19 (1.38-5.64)	0.819 (0.317-2.26)	0.801 (0.507-1.60)	2.97 (2.34-4.02)	n.a.	n.a.
92	m	0.352 (0.247-0.473)	6.08 (1.00-8.17)	0.252 (0.146-0.387)	16.08 (8.17-24.00)	3.87 (1.67-6.65)	0.935 (0.599-1.19)	1.10 (0.918-1.48)	3.72 (3.08-4.28)	0.80 (0.28-1.54)	1.41 (0.33-4.82)
183	m	0.432 (0.259-0.765)	2.50 (1.00-8.17)	0.165 (0.120-0.286)	24.00 (24.00-24.00)	5.58 (4.65-6.37)	1.20 (0.695-1.59)	0.977 (0.757-1.20)	3.41 (2.97-3.92)	0.95 (0.38-2.06)	1.67 (1.05-4.14)
Group 4 (3 x daily 40 µL of 0.15% CyclASol® per eye = 360 µg/day cyclosporine A)											
1	f	0.515 (0.146-1.03)	8.17 (1.00-24.00)	0.304 (0.113-0.727)	24.00 (24.00-24.00)	6.96 (3.02-9.94)	0.701 (0.360-1.24)	0.885 (0.489-1.37)	5.37 (2.06-8.45)	n.a.	n.a.
92	f	0.909 (0.331-2.26)	4.17 (0.17-8.17)	0.244 (0.104-0.445)	24.00 (24.00-24.00)	9.28 (4.07-12.4)	1.98 (0.563-3.70)	1.85 (0.976-2.97)	5.46 (2.53-7.99)	2.13 (0.41-4.98)	1.55 (0.71-3.81)
183	f	0.427 (0.374-0.493)	4.17 (0.00-8.17)	0.190 (0.123-0.252)	24.00 (24.00-24.00)	7.13 (6.21-8.70)	1.25 (0.875-1.64)	1.31 (1.00-1.66)	4.57 (3.97-5.41)	1.33 (0.37-3.38)	1.32 (0.72-2.17)
1	m	0.625 (0.267-1.11)	8.17 (1.00-8.17)	0.237 (0.108-0.325)	24.00 (24.00-24.00)	8.07 (4.42-13.6)	0.718 (0.192-1.74)	1.13 (0.478-2.02)	6.22 (2.59-9.86)	n.a.	n.a.
92	m	0.765 (0.393-1.20)	8.17 (1.00-8.17)	0.242 (0.142-0.358)	24.00 (24.00-24.00)	10.4 (6.42-16.0)	1.69 (0.733-3.42)	2.14 (1.08-4.20)	6.57 (3.97-9.54)	1.53 (0.52-4.23)	1.45 (0.69-2.44)
183	m	0.566 (0.297-0.828)	8.08 (1.00-8.17)	0.239 (0.123-0.328)	24.00 (24.00-24.00)	9.16 (4.78-13.4)	1.60 (0.626-2.78)	1.88 (0.982-2.72)	5.68 (3.17-7.93)	0.90 (0.53-1.46)	1.20 (0.71-1.70)

Arithmetic mean (range) toxicokinetic parameters of F4H5 in male and female New Zealand White rabbits after repeated ocular administrations of clinical CsA formulation

Day	Gender	C_{max} (ng/mL)	t_{max}[a] (h)	C_{last} (ng/mL)	t_{last}[a] (h)	AUC_{0-t} (h*ng/mL)	AUC_{0-4} (h*ng/mL)	AUC_{4-8} (h*ng/mL)	AUC_{8-24} (h*ng/mL)	RA C_{max}	RA AUC_{0-t}
Group 3 (3 x daily 40 µL of 0.05% CyclASol® per eye = 120 µg/day cyclosporine A)											
1	f	0.234 (0.105-0.338)	8.17 (1.00-8.17)	0.180 (0.105-0.261)	24.00 (8.17-24.00)	3.86 (0.429-6.04)	0.470 (0.103-0.824)	0.606 (0.309-0.793)	3.47 (2.75-4.76)	n.a.	n.a.
92	f	0.583 (0.145-2.30)	8.08 (4.17-24.00)	0.202 (0.145-0.315)	16.08 (8.17-24.00)	4.31 (0.897-10.5)	0.740 (0.389-0.957)	1.53 (0.486-5.16)	4.05 (2.98-4.71)	1.16 (0.68-2.11)	1.34 (0.26-3.78)
183	f	0.244 (0.146-0.334)	8.17 (4.17-8.17)	0.132 (0.109-0.177)	24.00 (24.00-24.00)	4.06 (3.16-5.09)	0.571 (0.242-0.775)	0.723 (0.540-1.00)	2.77 (2.12-3.46)	1.14 (0.69-1.94)	2.23 (0.80-7.65)
1	m	0.620 (0.254-1.50)	8.08 (0.00-8.17)	0.141 (0.107-0.190)	24.00 (8.17-24.00)	4.19 (1.38-5.64)	0.819 (0.317-2.26)	0.801 (0.507-1.60)	2.97 (2.34-4.02)	n.a.	n.a.
92	m	0.352 (0.247-0.473)	6.08 (1.00-8.17)	0.252 (0.146-0.387)	16.08 (8.17-24.00)	3.87 (1.67-6.65)	0.935 (0.599-1.19)	1.10 (0.918-1.48)	3.72 (3.08-4.28)	0.80 (0.28-1.54)	1.41 (0.33-4.82)
183	m	0.432 (0.259-0.765)	2.50 (1.00-8.17)	0.165 (0.120-0.286)	24.00 (24.00-24.00)	5.58 (4.65-6.37)	1.20 (0.695-1.59)	0.977 (0.757-1.20)	3.41 (2.97-3.92)	0.95 (0.38-2.06)	1.67 (1.05-4.14)
Group 4 (3 x daily 40 µL of 0.15% CyclASol® per eye = 360 µg/day cyclosporine A)											
1	f	0.515 (0.146-1.03)	8.17 (1.00-24.00)	0.304 (0.113-0.727)	24.00 (24.00-24.00)	6.96 (3.02-9.94)	0.701 (0.360-1.24)	0.885 (0.489-1.37)	5.37 (2.06-8.45)	n.a.	n.a.
92	f	0.909 (0.331-2.26)	4.17 (0.17-8.17)	0.244 (0.104-0.445)	24.00 (24.00-24.00)	9.28 (4.07-12.4)	1.98 (0.563-3.70)	1.85 (0.976-2.97)	5.46 (2.53-7.99)	2.13 (0.41-4.98)	1.55 (0.71-3.81)
183	f	0.427 (0.374-0.493)	4.17 (0.00-8.17)	0.190 (0.123-0.252)	24.00 (24.00-24.00)	7.13 (6.21-8.70)	1.25 (0.875-1.64)	1.31 (1.00-1.66)	4.57 (3.97-5.41)	1.33 (0.37-3.38)	1.32 (0.72-2.17)
1	m	0.625 (0.267-1.11)	8.17 (1.00-8.17)	0.237 (0.108-0.325)	24.00 (24.00-24.00)	8.07 (4.42-13.6)	0.718 (0.192-1.74)	1.13 (0.478-2.02)	6.22 (2.59-9.86)	n.a.	n.a.
92	m	0.765 (0.393-1.20)	8.17 (1.00-8.17)	0.242 (0.142-0.358)	24.00 (24.00-24.00)	10.4 (6.42-16.0)	1.69 (0.733-3.42)	2.14 (1.08-4.20)	6.57 (3.97-9.54)	1.53 (0.52-4.23)	1.45 (0.69-2.44)
183	m	0.566 (0.297-0.828)	8.08 (1.00-8.17)	0.239 (0.123-0.328)	24.00 (24.00-24.00)	9.16 (4.78-13.4)	1.60 (0.626-2.78)	1.88 (0.982-2.72)	5.68 (3.17-7.93)	0.90 (0.53-1.46)	1.20 (0.71-1.70)

Day	Gender	C_{max} (ng/mL)	t_{max}[a] (h)	C_{last} (ng/mL)	t_{last}[a] (h)	RA C_{max}
Group 4 (3 x daily 40 µL of 0.15% CyclASol® per eye = (b) (4) ng/day F4H5)						
1	f	45.5 (6.47-198)	4.17 (0.17-8.17)	45.5 (6.47-198)	4.17 (0.17-8.17)	n.a.
92	f	37.3 (5.64-73.4)	2.17 (0.17-8.17)	26.5 (5.64-71.5)	4.17 (0.17-8.17)	3.44 (0.028-7.13)
183	f	8.49 (5.81-11.2)	4.17 (0.17-8.17)	8.49 (5.81-11.2)	4.17 (0.17-8.17)	0.56 (0.56-0.56)
1	m	153 (5.31-527)	4.17 (0.17-8.17)	88.3 (5.31-527)	4.17 (0.17-8.17)	n.a.
92	m	38.3 (8.96-119)	0.17 (0.17-8.17)	23.3 (7.35-47.4)	6.17 (0.17-8.17)	4.34 (0.02-22.4)
183	m	26.7 (22.3-30.2)	4.17 (0.17-4.17)	19.6 (6.48-30.2)	4.17 (4.17-8.17)	2.51 (0.06-4.20)

a median (range); n.a. not applicable

Dosing Solution Analysis

- All dosing solutions remained within the specifications (± 5% nominal) throughout the treatment period of the study.

Nonclinical Toxicology of Novel Excipient F4H5

(See also previous reviews conducted under IND 128163)

Study title: 28 Day Toxicity Study with Oral Application of F4H5 in Wistar Han Rats

Study no.:	02G12028
Study report location:	4.2.3.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 24, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Perfluorobutylpentane (F4H5); 100209_D1_F1 + 5; 99.89%
Certificate of Analysis/Testing:	Yes

Key Study Findings:

- Ten Wistar Han rats per sex per group were dosed with 0, 500, 1000 or 2000 mg/kg/day F4H5 via oral gavage, daily for 28 days. An additional five rats per sex in the control and 2000 mg/kg/day groups were observed for a 28 day recovery period. CNS and respiratory safety pharmacology parameters were incorporated into the study.
- Changes in hematology parameters were observed at ≥500 mg/kg/day, including an decrease in white blood cell count (not recoverable) and decreased activated partial thromboplastin time (recoverable) in both sexes.
- Triglyceride levels were significantly increased at ≥1,000 mg/kg/day in both sexes (partially recoverable).
- Changes in urinalysis parameters were observed at 2,000 mg/kg/day in both sexes (not recoverable).
- The NOAEL in this study was (b) (4) mg/kg/day, providing an exposure margin of 95X based on the dose of F4H5 in proposed clinical trial of (b) (4) mg/kg, on a mg/m^2 basis.

The C_{max} corresponding to 500 mg/kg/day was (b) (4) ng/mL, which provides an exposure margin of 160X.

Methods:

Doses:	0,500,1000 and 2000 mg/kg
Frequency of dosing:	Daily for 28 days
Route of administration:	Oral
Dose volume:	0.39-2 mL/kg
Formulation/Vehicle:	0.9%NaCl
Species/Strain:	Wistar Han rats
Number/Sex/Group:	10 rats/sex/group; an additional 5 rats/sex/group were sacrificed after a 4 week recovery period in the control and high dose groups; an additional 6rats/sex/group were used for toxicokinetic analysis (3 rats/sex/group for the recovery control and non-recovery groups)
Age:	Approximately 4-6 weeks old
Weight:	230- 243 g males; 165-176 g females
Satellite groups:	None
Unique study design:	The study included a functional observational battery, locomotor activity and whole body plethysmography as safety pharmacology parameters. An additional 4 rats/sex/group were sacrificed on Day 0 and Day 27 for the lung function assay.
Deviation from study protocol:	None that would affect the results of the study

Observations and Results

Proposed observations and times:

Mortality:	Daily
Clinical signs:	Daily; detailed-weekly
Food consumption:	Pretest and twice weekly
Water consumption:	Pretest and twice weekly
Body weights:	Pretest and twice weekly
Ophthalmoscopy:	Pretest and day 28
Locomotor activity:	Day 28
Functional observational battery:	Day 1 and 28
Lung function measurements:	Day 1 and 28
Hematology:	Pretest, day 28 and 56 (recovery)
Clinical chemistry:	Pretest, day 28 and 56 (recovery)

Urinalysis:	Pretest, day 28 and 56 (recovery)
Organ weights:	Day 28 and 56 (recovery)
Histopathology:	Day 28 and 56 (recovery)
Toxicokinetics:	Days 0 and 27

Mortality (Daily)- There were no unscheduled deaths.

Clinical Signs (Daily; detailed-weekly)- No test article-related effects were observed.

Food consumption (Pretest and twice weekly)- No test article-related effects were observed.

Water consumption (Pretest and twice weekly)- No test article-related effects were observed.

Body Weights (Pretest and twice weekly)- No test article-related effects were observed.

Ophthalmoscopy (Pretest and day 28)- Indirect and slit lamp ophthalmoscopy were performed. No test article-related effects were observed.

Locomotor activity (Day 28)- Number of rearings was significantly increased during one 15 min interval out of four total, in females dosed with 500 mg/kg (48%) and 1000 mg/kg (+56%). Only the 1000 mg/kg value reached statistical significance ($p<0.05$) and the effect was not observed in the highest dose (2000 mg/kg/day) nor in male animals. Also, time in rest was significantly increased (+9%) and time in movement significantly decreased (-46%) in males dosed with 1000 mg/kg, as measured in one interval. The effect was not observed in females. Due to the inconsistency of these effects, lack of dose response, and presence in one sex, this reviewer concluded the results did not show a treatment-related effect.

Functional observational battery (Day 1 and 28)- No test article-related effects were observed.

Lung function measurements (Day 1 and 28)- A decrease in inspiratory time at 15-20 min after treatment with 1000 mg/kg (31%) and 2000 mg/kg (26%) F4H5 was the only statistically significant difference compared to the control group on Day 0 only (males and females combined). Measurements were taken up to 180 minutes post-dose (eight time points considered) and no other deviations were observed. As such, the effect was considered incidental and not treatment related.

Hematology (Pretest, day 28 and 56 [recovery])- A significant decrease in white blood cell counts was observed in females at all doses tested. The effect was not recoverable. Activated partial thromboplastin time (aPTT) was significantly decreased in males and females; however this effect was not observed after recovery.

Percent Change in Hematology Parameters (Week 4 and 8)

	Dose (mg/kg)			
Parameter	**500**	**1000**	**2000**	**2000**

				+recovery (week 8)
WBC (G/L) m/f	-22/-26**	-29/-35**	-29/-40**	-20*/-22*
aPTT m/f	-	-19**/-19**	-25**/-28**	-

*P<0.05; **P<0.01

Clinical Chemistry (Pretest, day 28 and 56 [recovery]) - A significant increase in triglycerides was observed at ≥1000 mg/kg in both sexes. The effect was not fully recoverable.

Percent Change in Clinical Chemistry Parameters (Week 4 and 8)

	Dose (mg/kg)			
Parameter	**500**	**1000**	**2000**	**2000 +recovery (week 8)**
Triglycerides (mmol/L) m/f	-	+109**/+90**	+33/+56*	+26/+8

*P<0.05; **P<0.01

Urinalysis (Pretest, day 28 and 56 [recovery])- A statistically significant decrease in urine sodium and chloride levels was observed at the 2000 mg/kg dose and these effects were not recoverable.

Percent Change in Urinalysis Parameters (Week 4 and 8)

	Dose (mg/kg)			
Parameter	**500**	**1000**	**2000**	**2000 +recovery (week 8)**
Urine sodium (mmol/L) m/f	-	-	-25**/-28*	-/-28**
Urine chloride (mmol/L) m/f	-	-	-48**/-49**	-17/-43**

*P<0.05; **P<0.01

Organ weights (Day 28 and 56 [recovery])- No test article-related effects were observed.

Histopathology (Day 28 and 56 [recovery])

Adequate Battery- Yes

Signed Pathology Report – Yes

Peer Review- No

Macroscopic Findings: No test article related effects were observed with the exception of discoloration (dark red) of the extraorbital lacrimal gland in male #3109 dosed with 1000 mg/kg/day F4H5.

Microscopic Findings: In most cases, only the control and high dose animals were evaluated. Atrophy of the harderian gland was observed in one male and one female at 2000 mg/kg. Inflammatory cell infiltration in the harderian gland was observed in males and females at 2000 mg/kg. Inflammatory infiltration was also observed in the kidney males and females at ≥1000 mg/kg (Table 5).

Inflammatory infiltration was observed in the harderian gland in recovery animals, including two male controls (out of 5; slight), two high dose (2000 mg/kg) males (out of 5; slight to moderate) and one female control (out of 5; very slight).

This reviewer noted these findings but did not consider them test article-related.

Table 5: Microscopic Findings:

Tissue/finding	# examined & severity	Males				Females			
Dose (mg/kg)		0	500	1000	2000	0	500	1000	2000
Harderian gland	# examined	10	0	0	10	10	0	0	10
Atrophy	Very slight	0	-	-	1	0	-	-	0
	Slight	0	-	-	1	0	-	-	1
Inflammatory cell infiltration	Very slight	0	-	-	3	0	-	-	3
	Slight	0	-	-	2	0	-	-	0
	Moderate	0	-	-	1	0	-	-	0
Kidney	# examined	10	10	10	10	10	10	10	10
Inflammatory cell infiltration	Very slight	0	0	2	1	0	0	0	1

Toxicokinetics (Days 0 and 27) - Blood samples were taken 8 min to 72 hours postdose on Day 0 and 27. Mean terminal half-lives ($t_{1/2}$) could not be calculated at day 0 in male rats and ranged between 86.9 to 146.8 hours on treatment day 27 (Table 6). Terminal half-life in females ranged between 6.6 to 11.6 hours on treatment day 0 and between 68.6 to 123.7 h on treatment day 27 (Table 7). Dose normalized AUC-values as well as dose normalized C_{max} values on treatment day 0 and day 27 indicate no dose linearity after treatment of male and female rats with F4H5 at 500, 1000, and 2000 mg/kg/day.

Table 6: Toxicokinetic Parameters of F4H5 in Rats-Males

Day	Parameter	Units	500 mg/kg	1000 mg/kg	2000 mg/kg
Day 0	AUC_{last}	h*ng/ml	15861.7	12344.6	25561.3
	AUC_{last} /D	h*ng/ml/mg	31.8	12.3	12.8
	t_{max}	h	8	6	6
	C_{max}	ng/ml	1872	1063	2291
	C_{max} /D	ng/ml/mg	3.7	1.1	1.1
	$t_{1/2}$	h	n.c.	n.c.	n.c.
	Lz	1/h	n.c.	n.c.	n.c.
Day 27	AUC_{last}	h*ng/ml	17723.4	12189.3	18453.3
	AUC_{last} /D	h*ng/ml/mg	35.4	12.2	9.2
	t_{max}	h	8	6	1
	C_{max}	ng/ml	1161	620	1160
	C_{max} /D	ng/ml/mg	2.3	0.6	0.5
	$t_{1/2}$	h	86.9	146.6	125.6
	Lz	1/h	0.008	0.005	0.006

$AUC_{last} = AUC_{0-24h}$, $t_{1/2}$ = half life
/D = normalized to 1 mg/d, Lz = elimination constant, n.c. = not calculated

Table 7: Toxicokinetic Parameters of F4H5 in Rats-Females

Day	Parameter	Units	500 mg/kg	1000 mg/kg	2000 mg/kg
Day 0	AUC_{last}	h*ng/ml	4912.2	9166.9	16337
	AUC_{last} /D	h*ng/ml/mg	9.8	9.2	8.2
	t_{max}	h	2	2	2
	C_{max}	ng/ml	690	1090	1883
	C_{max} /D	ng/ml/mg	1.4	1.1	0.9
	$t_{1/2}$	h	6.6	11.2	11.6
	Lz	1/h	0.105	0.062	0.06
Day 27	AUC_{last}	h*ng/ml	13341.1	13452.7	25179.6
	AUC_{last} /D	h*ng/ml/mg	26.7	13.5	12.6
	t_{max}	h	1	1	1
	C_{max}	ng/ml	859	1315	1929
	C_{max} /D	ng/ml/mg	1.7	1.3	1.0
	$t_{1/2}$	h	123.7	110.3	68.6
	Lz	1/h	0.006	0.006	0.01

$AUC_{last} = AUC_{0-24h}$, $t_{1/2}$ = half life
/D = normalized to 1 mg/d, Lz = elimination constant, n.c. = not calculated

Excerpted from the sponsor's study report, page 134

Stability and homogeneity: Stability of the stock test item was confirmed under room temperature for 5 weeks. Stability of the working solutions was confirmed under room temperature for 4 weeks. The sponsor will be asked to provide dose analysis data.

Study title: 26 Week Toxicity Study with Oral Application of F4H5 in Wistar Han Rats

Study no.:	02G12028
Study report location:	4.2.3.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 15, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Perfluorobutylpentane (F4H5); 12174417; 99.7%
Certificate of Analysis/Testing:	Yes

Key Study Findings:

- Twenty male and female Wistar Han rats were dosed with 0, 250, 500 or 1000 mg/kg/day F4H5 via oral gavage, daily for six months. An additional four rats per sex in the control and 1000 mg/kg/day groups were observed for a 28 day recovery period.
- There were four unscheduled deaths, including one in the control group, two in the 250 mg/kg/day group and one in the 1000 mg/kg/day group. It is unclear if the deaths were test article related, as histopathology analysis did not reveal a cause of death and deaths were not dose-dependent. The reviewer considers the death at 1000 mg/kg/day to be test article related due to adverse clinical signs before death.
- Hematology (decreased white blood cell counts), clinical chemistry (gamma glutamyltransferase, alkaline phosphatase, triglycerides) and urinalysis (urine weight, osmolarity and sodium) parameters were affected starting at ≥250 mg/kg/day in both sexes; changes urinalysis parameters (weight, osmolarity and sodium) were generally limited to females. aPTT was decreased at ≥500 mg/kg/day.
- A NOAEL was not identified in this study. The lowest observable adverse effect (LOAEL) was (b) (4) mg/kg/day, which is 48X the proposed clinical dose, on a mg/m² basis. The C_{max} corresponding to (b) (4) mg/kg/day was (b) (4) ng/mL, which provides an exposure margin of 160X.
- The exposure margin for mortality at (b) (4) mg/kg is 190X based on mg/m² and 125X based on C_{max} values.

Methods:

Doses:	0, 250,500,1000 mg/kg
Frequency of dosing:	Daily for 26 weeks
Route of administration:	Oral
Dose volume:	0.19-1 mL/kg
Formulation/Vehicle:	0.9%NaCl
Species/Strain:	Wistar Han rats
Number/Sex/Group:	20 rats/sex/group; an additional 10 rats/sex/group were sacrificed after a 4 week recovery period in the control and high dose groups; an additional 3 rats/sex/group were used for toxicokinetic analysis (non-recovery groups only); 7 rats/sex/group served as sentinel animals
Age:	Approximately 4-6 weeks old
Weight:	190- 217 g males; 145- 157 g females
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that would affect the results of the study

Observations and Results

Proposed observations and times:

Mortality:	Daily
Clinical signs:	Daily; detailed-weekly
Food consumption:	Pretest and twice weekly
Water consumption:	Pretest and twice weekly
Body weights:	Pretest and twice weekly
Ophthalmoscopy:	Pretest, day 182 and 210 (recovery)
Hematology:	Pretest, day 91, 182 and 210 (recovery)
Clinical chemistry:	Pretest, day 91, 182 and 210 (recovery)
Urinalysis:	Day 91, 182 and 210 (recovery)
Organ weights:	Day 182 and 210 (recovery)
Histopathology:	Day 182 and 210 (recovery)
Toxicokinetics:	Day 0 and 180

Mortality (Daily)- There were four unscheduled deaths:

- Group 1 (control):
  Male 1114, day 13, found dead
  Gross pathology: without visible lesions.
- Group 2 (250 mg/kg F4H5):
  Male 2103, day 95, killed moribund.
  Gross pathology: exophthalmos following blood collection
  Female 2214, day 91, killed moribund.
  Gross pathology: exophthalmos following blood collection.
- Group 4 (1000 mg/kg F4H5):
  Female 4205, day 92, killed moribund.
  Clinical observation: bad general condition, lethargy, severe body weight reduction.
  Gross pathology: lung with dark red spots; dilatation of the left cardiac atrium.

The sponsor did not provide any further explanation and deemed the deaths not test article-related. Histopathological evaluation did not provide a cause of death. This reviewer considers the death at 1000 mg/kg/day to be test article-related due to adverse preclinical clinical signs before death. It is unclear if death was related to treatment in the TK animals at 250mg/kg.

Clinical Signs (Daily; detailed-weekly)- No test article-related effects were observed.

Food consumption (Pretest and twice weekly)- No test article-related effects were observed.

Water consumption (Pretest and twice weekly)- No test article-related effects were observed.

Body Weights (Pretest and twice weekly)- No test article-related effects were observed.

Ophthalmoscopy (Pretest, day 182 and 210 [recovery])- Indirect and slit lamp ophthalmoscopy were performed. No test article-related effects were observed.

Hematology (Pretest, day 91, 182 and 210 [recovery])- A significant decrease in white blood cell counts was observed in males and females. Activated partial thromboplastin time (aPTT) was significantly decreased in males and females. These effects were also observed after recovery.

Percent Change in Hematology Parameters (Week 26 and 30 [recovery])

	Dose (mg/kg)			
Parameter	**250**	**500**	**1000**	**1000 +recovery (day 210)**
WBC (G/L) (m/f)	-22/-26**	-29/-35**	-29/-40**	-17*/22*
aPTT (m/f)	-	-/-12**	-8.8**/-14**	-7*/-19**

*P<0.05; **P<0.01

Clinical Chemistry (Pretest, day 91, 182 and 210 [recovery])- An increase in gamma glutamyl transferase (GGT) and alkaline phosphatase (AP) levels was observed at all doses at the end of the main study (week 26). The effect was not fully recoverable. GGT levels were also elevated between 6X and 9.5X on week 13 (Day 91) but the response was not dose dependent. Without a pathological correlate, the significance of these findings is unclear.

Triglycerides were elevated in both sexes at all does (although not significant in female main study) and the effect was not recoverable. Triglyceride elevation was also observed in the 28-day oral toxicology study in males and females.

Percent Change in Clinical Chemistry Parameters (Day 182 and 210 [recovery])

	Dose (mg/kg)			
Parameter	**250**	**500**	**1000**	**1000 +recovery (day 210)**
GGT (U/L) m/f	+340**/+200	+400**/+300*	+480*/+266	+36/+300
AP (U/L) m/f	+32*/+86**	-/ +108	+27*/+115**	+25*/+60**
Triglycerides (mmol/L)	+40*/+10	+33*/+20	+48**/+20	+37/+41*

*P<0.05; **P<0.01

Urinalysis (Day 91, 182 and 210 [recovery])- Urine weight was increased at all doses in females and the effect was not recoverable. Urine osmolarity was decreased in females at all doses and the effect was partially recoverable. Urine sodium was decreased at the highest dose of 1000 mg/kg in males and females. These changes indicate a possible diuretic effect, however they were not observed after the recovery

period. Without changes in other systems like renal function, the biological significance of these findings is unclear.

Percent Change in Urinalysis Parameters (Day 182 and 210)

	Dose (mg/kg)			
Parameter	**250**	**500**	**1000**	**1000 +recovery (day 210)**
Urine weight (g) m/f	-/+103*	-/+153**	-/+173**	-/+38
Urine osmolarity (Osmol/L) m/f	-/-30*	-/-33**	-/-41**	-/-11
Urine sodium (mmol/L) m/f	-/-	-/-	-21*/-58**	-/-

*P<0.05; **P<0.01

Organ weights (Day 182 and 210 [recovery])- No test article-related effects were observed.

Histopathology (Day 182 and 210 [recovery])

Adequate Battery- Yes

Signed Pathology Report – Yes

Peer Review- No

Macroscopic Findings: No test article-related effects were observed.

Microscopic Findings:

Non-recoverable effects:
Inflammatory cell infiltration was observed at a higher incidence in the eyes of males and females dosed with 1000 mg/kg F4H5 than controls. Infiltrate was also observed in the harderian gland in animals dosed with 250 or 1000 mg/kg. Lipidosis of the liver was observed in males and females at ≥500 mg/kg (Table 8).

Recoverable effects:
Thickening of the basement membrane of the tubuli in the kidney was observed at all doses in males, but not females. Lastly, necrosis in the fat of the mesentery and steatitis were observed at 500 mg/kg in males and the effect was recoverable (Table 8).

Effects in recovery animals:
Single cell necrosis in the liver was observed in male and female 1000 mg/kg recovery animals only (Table 8).

This reviewer noted these findings but did not consider them test article- related.

Table 8: Microscopic Findings

Tissue/finding	# examined & severity	Male						Female					
Dose (mg/kg)		0	0+r	250	500	1000	1000+r	0	0+r	250	500	1000	1000+r
Eye	# examined	20	10	1	0	20	10	20	10	1	0	20	10
Inflammatory cell infiltration	Very slight	0	0	0	-	0	0	0	0	0	-	1	0
	Slight	1	0	0	-	3	1	1	1	0	-	3	1
Harderian gland	# examined	20	10	1	0	20	10	20	10	1	0	20	10
Inflammatory cell infiltration	Very slight	0	0	0	-	0	2	1	1	0	-	0	0
	Slight	1	0	0	-	2	5	0	1	0		3	1
	Moderate	0	0	0	-	1	0	0	0	0	-	0	0
	Severe	0	0	1	-	0	0	0	0	0	-	0	0
Kidney	# examined	20	10	20	20	20	10	20	10	20	20	20	10
Thickening of basement membrane, tubuli	Very slight	0	0	1	1	3	0	0	1	0	0	0	0
	Slight	0	0	0	1	0	0	0	0	0	0	0	0
Liver	# examined	20	10	20	20	20	10	20	10	20	20	20	10
Lipidosis	Very slight	0	0	0	0	1	0	0	0	0	0	2	1
	Slight	0	0	0	0	0	0	0	0	0	1	0	2
Single cell necrosis	Very slight	0	0	0	0	0	3	0	0	0	0	0	1
Mesentery	# examined	20	10	1	2	20	10	20	10	1	0	20	10
Necrosis in fat	Moderate	0	0	0	2	0	0	0	0	0	-	0	0
Steatitis	Moderate	0	0	0	2	0	0	0	0	0	-	0	0

Toxicokinetics (Day 0 and 180)- High systemic exposure was achieved for all dose levels. Significant increases (generally 2 to 3-fold) in systemic exposure, based on AUC, were observed for both sexes from treatment day 0 to day 180 (Tables 9 and 10). Increases in AUC were subproportionate, and approximately dose proportionate from the mid to the high dose. Plasma C_{max} values were similar (range: 437 – 1150 ng/mL) for all dose groups and both sexes, and were also similar between the Day 0 and Day 180 intervals. T_{max} was generally attained at 2 to 4 hours after dosing at all doses and intervals, though there was some variability. $T_{1/2}$ was similar for all dose levels and for both sexes within an interval, but was significantly greater at the Day 180 interval

Day	Parameter	Units	250 mg/kg	500 mg/kg	1000 mg/kg
Day 0	AUC_{last}	h*ng/ml	2768.5	7396.3	6094.3
	AUC_{last} /D	h*ng/ml/mg	11.1	14.8	6.1
	t_{max}	h	2	2	2
	C_{max}	ng/ml	437	1092	741
	C_{max} /D	ng/ml/mg	1.7	2.2	0.7
	$t_{1/2}$	h	5.7	5.2	7.4
	Lz	1/h	0.122	0.133	0.094
Day 180	AUC_{last}	h*ng/ml	13170.8	9671.4	18777.4
	AUC_{last} /D	h*ng/ml/mg	52.7	19.3	18.8
	t_{max}	h	4	2	2
	C_{max}	ng/ml	1150	785	907
	C_{max} /D	ng/ml/mg	4.6	1.6	0.9
	$t_{1/2}$	h	23	22.5	27
	Lz	1/h	0.03	0.031	0.026

Day 0: AUC_{last} = AUC_{0-24h}, Day 180: AUC_{last}=AUC_{0-168h}, $t_{1/2}$ = half life
/D = normalized to 1 mg/d, Lz = elimination constant, n.c. = not calculated
For toxicokinetic calculations values <LOQ were considered for calc. with 0.5 LOQ
Excerpted from the sponsor's study report, page 212

Stability: The stock solutions were stable for at least five weeks, the working standard solutions for 15 days at 5 – 10 °C. The sponsor will be asked to provide dose analysis data.

(22.5 – 28.3 h) as compared with Day 0 (4.7 – 7.4 h). Dose normalized AUC-values as well as dose normalized C_{max} values on treatment day 0 and day 180 indicated no dose linearity as well as no differences between males and females after treatment with F4H5 at 250, 500, and 1000 mg/kg/day F4H5.

Table 9: Toxicokinetic parameters of F4H5- Males

Day	Parameter	Units	250 mg/kg	500 mg/kg	1000 mg/kg
Day 0	AUC_{last}	h*ng/ml	4228.6	5396.4	12629
	AUC_{last} /D	h*ng/ml/mg	16.9	10.8	12.6
	t_{max}	h	2	2	4
	C_{max}	ng/ml	696	940	887
	C_{max} /D	ng/ml/mg	2.8	1.9	0.9
	$t_{1/2}$	h	4.7	5.0	n.c.
	Lz	1/h	0.149	0.138	n.c.
Day 180	AUC_{last}	h*ng/ml	9146.3	14295.6	26576.6
	AUC_{last} /D	h*ng/ml/mg	36.6	28.6	26.6
	t_{max}	h	0.5	4	4
	C_{max}	ng/ml	547	806	992
	C_{max} /D	ng/ml/mg	2.2	1.6	1.0
	$t_{1/2}$	h	26.2	24.8	28.3
	Lz	1/h	0.027	0.028	0.025

Day 0: AUC_{last} = AUC_{0-24h}, Day 180: AUC_{last}=AUC_{0-168h}, $t_{1/2}$ = half life
/D = normalized to 1 mg/d, Lz = elimination constant, n.c. = not calculated
For toxicokinetic calculations values <LOQ were considered for calc. with 0.5 LOQ
Excerpted from the sponsor's study report, page 212

Table 10: Toxicokinetic parameters of F4H5- Females

Day	Parameter	Units	250 mg/kg	500 mg/kg	1000 mg/kg
Day 0	AUC_{last}	h*ng/ml	2768.5	7396.3	6094.3
	AUC_{last} /D	h*ng/ml/mg	11.1	14.8	6.1
	t_{max}	h	2	2	2
	C_{max}	ng/ml	437	1092	741
	C_{max} /D	ng/ml/mg	1.7	2.2	0.7
	$t_{1/2}$	h	5.7	5.2	7.4
	Lz	1/h	0.122	0.133	0.094
Day 180	AUC_{last}	h*ng/ml	13170.8	9671.4	18777.4
	AUC_{last} /D	h*ng/ml/mg	52.7	19.3	18.8
	t_{max}	h	4	2	2
	C_{max}	ng/ml	1150	785	907
	C_{max} /D	ng/ml/mg	4.6	1.6	0.9
	$t_{1/2}$	h	23	22.5	27
	Lz	1/h	0.03	0.031	0.026

Day 0: AUC_{last} = $AUC_{0\text{-}24h}$, Day 180: AUC_{last}=$AUC_{0\text{-}168h}$, $t_{1/2}$ = half life
/D = normalized to 1 mg/d, Lz = elimination constant, n.c. = not calculated
For toxicokinetic calculations values <LOQ were considered for calc. with 0.5 LOQ

Excerpted from the sponsor's study report, page 212

Study title: 28 Day Toxicity Study with F4H5 in New Zealand White Rabbits After Repeated Administration into the Conjunctival Sac

Study no.:	20-3-0106-12
Study report location:	4.2.3.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 14, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	F4H5;Batch 100209 D1 F3+F4; purity-99.91%
Certificate of Analysis/Testing:	Yes

Key Study Findings:

- Six male and female New Zealand White rabbits received 0, 513, 1026 or 1538 mg/day F4H5 (total for both eyes) to the conjunctival sac for 28 days. An additional four rabbits per sex, in the control and 1538 mg/day groups were observed for a 28 day recovery period.
- One male in the 513 mg/day group died on Day 0 after conflict with his cage partner and this death was not considered treatment-related.
- Cardiovascular safety pharmacology parameters were included in this study. The only significant change was an increased heart rate at all doses in females on Day 28, which was transient and not observed after recovery, on Day 55.
- Activated partial thromboplastin time was shortened at all doses in females and the effect was not recoverable.
- A NOAEL was not identified in this study. The LOAEL (based on aPTT) in this study was (b) (4) mg/day, corresponding to an exposure margin of 108X, based on the proposed clinical starting dose of (b) (4) mg/kg, on a mg/m² basis. The C_{max} corresponding to (b) (4) mg/kg/day was (b) (4) ng/mL, which provides an exposure margin of 52X.

Methods:	
Doses:	0, 513, 1026 or 1538 mg/day
Frequency of dosing:	Daily for 28 days
Route of administration:	Administration into the conjunctival sac
Dose volume:	200 µl, once, twice or three times per day to each eye for a total of 513, 1026 and 1538 mg F4H5/animal/day.
Formulation/Vehicle:	0.9% NaCl
Species/Strain:	New Zealand White Rabbit
Number/Sex/Group:	6 rabbits/sex/group; an additional 4 rabbits/sex/group were sacrificed after a 28 day recovery period in the control and high dose groups
Age:	2-3 months old
Weight:	2.2- 3.1 kg both sexes
Satellite groups:	None
Unique study design:	Cardiovascular safety pharmacology parameters (ECG) were evaluated as part of this study.
Deviation from study protocol:	None that would affect the results of the study.

Observations and Results

Proposed observations and times:

Mortality:	Daily
Clinical signs:	Pretest and weekly
Food consumption:	Pretest and weekly
Body weights:	Pretest and weekly
Ophthalmoscopy:	Pretest, day 28 and 55 (recovery)
Electrocardiogram:	Pretest, day 28 and 55 (recovery)
Hematology:	Pretest, day 28 and 55 (recovery)
Clinical chemistry:	Pretest, day 28 and 55 (recovery)
Urinalysis:	Day 28 and 55
Organ weights:	Day 28 and 55
Histopathology:	Day 28 and 55
Toxicokinetics:	Day 27 and 28

Mortality (Daily)- Male 206 (513 mg/day F4H5) died shortly after conflict with his cage partner on Day 0 after administration of the first dose. This animal was subjected to a complete gross necropsy and a complete list of tissues was collected for possible histopathological examination. During necropsy several bite wounds were noted at the ear, neck, back and limbs. In addition, an extensive hematoma was found in the lower jaw and ventral neck area which was considered to be due to blood collection from the V. jugularis. As such, the death was not considered test-article related. There were no other unscheduled deaths.

Clinical Signs (Pretest and weekly)- No test article-related effects were observed.

Food consumption (Pretest and weekly)- No test article-related effects were observed.

Body Weights (Pretest weekly)- No test article-related effects were observed.

Ophthalmoscopy (Pretest, day 28 and 55 [recovery])- Conjunctival hyperemia was observed on Day 28 and Day 55 in one male dosed with 1538 mg/day. Slight reddening of the conjunctiva was observed in one male on Day 55 (1538 mg/day; recovery). Pale conjunctiva/iris was observed in one male dosed with 513 mg/day on Day 28. There were no other ophthalmic observations.

Electrocardiogram (Pretest, day 28 and 55 [recovery])- On Day 28, heart rate was significantly higher in females dosed with 513 mg/day (+20%; $p \leq 0.05$) or 1026 mg/day (+18%; $p \leq 0.05$). Heart rate of females dosed with 1538 mg/day was also higher than controls (+10%) but this difference was not statistically significant.

Hematology (Pretest, day 28 and 55 [recovery])- Activated partial thromboplastin time (aPTT) time was decreased at all doses (-22%, -25% and -21% at 513 mg/day, 1026 mg/day and 1538 mg/day, respectively; $p<0.05$) in females only, on Day 28. The decrease

was also observed after recovery, in females dosed with 1538mg/day (-23%; not statistically significant).

Clinical Chemistry (Pretest, day 28 and 55 [recovery])- No test article-related effects were observed.

Urinalysis (Day 28 and 55)- No test article-related effects were observed.

Organ weights (Day 28 and 55)- No test article-related effects were observed.

Histopathology (Day 28 and 55)

Adequate Battery- Yes

Signed Pathology Report – Yes

Peer Review- No

Macroscopic Findings: No test article-related effects were observed. 513 1026 1538

Microscopic Findings: Congestion in the liver was observed at ≥513 mg/day in males and females. In the lungs, granulocytic cell infiltration was observed at a higher frequency in test-article treated animals than controls. Mineralization was observed at ≥513mg/day in males and females. Rathke's cleft dilation was observed at a higher frequency and severity in test-article treated animals (≥1026 mg/day) than controls. Lastly, epithelial mineralization of the stomach was observed at ≥513 mg/day in females (Table 11).

Histopathology was not assessed in recovery animals.

This reviewer noted these findings but did not consider them test article- related.

Table 11: Microscopic findings (Day 28):

Tissue/finding	# examined & severity	Males				Females			
Dose (mg/day)		0	513	1026	1538	0	513	1026	1538
Liver	# examined	6	6	6	6	6	6	6	6
Congestion	Grade 2	0	0	1	0	0	2	0	1
Lungs	# examined	6	6	6	6	6	6	6	6
Granulocytic cell infiltration	Grade 1	0	3	2	2	1	5	3	3
	Grade 2	0	1	0	0	1	0	1	1
Mineralization	Grade 1	0	1	1	1	0	0	1	1
Pituitary gland	# examined	6	6	6	6	6	6	6	6
Rathke's cleft dilation	Grade 1	1	0	2	1	0	0	2	2
	Grade 2	0	0	0	1	0	0	0	0
Stomach	# examined	6	6	6	6	6	6	6	6
Epithelial mineralization	Grade 1	0	0	0	0	0	1	0	2

Toxicokinetics (Days 27 and 28)- Toxicokinetic investigations revealed a generally very low systemic exposure of the animals to F4H5 at all doses levels (Table 12). Inter-individual variability was generally high at all dose levels and over the duration of treatment. In male and female rabbits mean maximum blood concentrations (C_{max}) were attained very early at the first measured time point (0.13 h) at the beginning as well as at the end of the treatment period. Half-life ranged between 6.3 and 13.1 hours on Day 0 and 7.2 to 21.2 hours on Day 27. Absolute AUC-values as well as C_{max} values on treatment day 0 and day 27 were similar in all dose groups indicating similar systemic exposure in all dose groups. Exposure was similar in all dose groups even though the animals of the low, mid and high dose groups received one, two or three of administrations of test-article per day, respectively.

Table 12: Toxicokinetic parameters of F4H5 after subconjunctival administration to rabbits- Males (top) and Females (bottom)

Day	Parameter	Units	513 mg/d	1026 mg/d	1538 mg/d
Males (Mean Values)					
Day 0	AUC_{last}	h*ng/mL	722	349	282
	AUC_{last} /D	h*ng/mL/mg	1.4	0.3	0.2
	T_{max}	h	0.13	0.13	0.13
	C_{max}	ng/mL	479	328	314
	C_{max} /D	ng/mL/mg	0.9	0.3	0.2
	$T_{1/2}$	h	5.8	6.9	n.c.
	λ_z	1/h	0.12	0.1	n.c.
Day 27	AUC_{last}	h*ng/mL	617	492	512
	AUC_{last} /D	h*ng/mL/mg	1.2	0.4	0.3
	T_{max}	h	0.13	0.13	0.13
	C_{max}	ng/mL	574	287	198
	C_{max} /D	ng/mL/mg	0.7	0.3	0.1
	$T_{1/2}$	h	16.9	21.2	12.6
	λ_z	1/h	0.04	0.03	0.05
Females (Mean Values)					
Day 0	AUC_{last}	h*ng/mL	477	362	136
	AUC_{last} /D	h*ng/mL/mg	0.9	0.3	0.5
	T_{max}	h	0.13	0.13	0.13
	C_{max}	ng/mL	245	441	1178
	C_{max} /D	ng/mL/mg	0.4	0.4	0.7
	$T_{1/2}$	h	7.7	6.3	13.1
	λ_z	1/h	0.09	0.11	0.05
Day 27	AUC_{last}	h*ng/mL	555	655	376
	AUC_{last} /D	h*ng/mL/mg	1.0	0.6	0.6
	T_{max}	h	0.13	0.13	0.13
	C_{max}	ng/mL	358	391	144
	C_{max} /D	ng/mL/mg	0.6	0.7	0.2
	$T_{1/2}$	h	9.5	7.2	8.6
	λ_z	1/h	0.07	0.1	0.08

Excerpted from the sponsor's study report, pages 402 and 403

Stability and homogeneity: The stock solutions were confirmed stable for at least five weeks, the working standard solutions for 15 days at 5 – 10 °C. The sponsor will be asked to provide dose analysis data.

6 Months Toxicity Study with F4H5 in New Zealand White Rabbits After Repeated Administration into the Conjunctival Sac

Study no.:	20-3-0120-12
Study report location:	4.2.3.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 24, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	F4H5;Batch 120075 P1; purity-99.8%
Certificate of Analysis/Testing:	Yes

Key Study Findings:

- Six male and female New Zealand White rabbits received 0, 513, 1026 or 1538 mg/day F4H5 (total for both eyes) to the conjunctival sac for six months daily. An additional four rabbits per sex, in the control and 1538 mg/day groups were observed for a 28 day recovery period.
- One female in the 1538 mg/day recovery group was found dead on Day 131. The death was not considered test article-related.
- The P wave was significantly longer in males dosed with 1026 mg/day on Day 28 however this change was transient and no other ECG changes were observed (including none in the highest dose group of 1538 mg/day).
- Activated partial thromboplastin time was significantly decreased (approx. 13 -32%) in females ≥513 mg/day, however the effect was not observed in recovery animals.
- Glutamate dehydrogenase and triglycerides were increased in recovery males dosed with 1538 mg/day F4H5.
- A NOAEL was not identified in this study. The LOAEL in this study was (b) (4) mg/day, corresponding to an exposure margin of 108X, based on the proposed clinical starting dose of (b) (4) mg/kg, on a mg/m^2 basis. The C_{max} corresponding to (b) (4) mg/kg/day was (b) (4) ng/mL, which provides an exposure margin of 34X.

Pathology)

Methods:	
Doses:	0, 513, 1026 or 1538 mg/day
Frequency of dosing:	Daily for 26 weeks
Route of administration:	Administration into the conjunctival sac
Dose volume:	200 µl, once, twice or three times per day to each eye for a total of 513, 1026 and 1538 mg F4H5/animal/day.
Formulation/Vehicle:	0.9% NaCl
Species/Strain:	New Zealand White Rabbit
Number/Sex/Group:	6 rabbits/sex/group; an additional 4 rabbits/sex/group were sacrificed after a 28 day recovery period in the control and high dose groups
Age:	2-3 months old
Weight:	2.1- 3.4 kg both sexes
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that would affect the results of the study.

Observations and Results

Proposed observations and times:

Mortality:	Daily
Clinical signs:	Pretest and days 7, 14, 28, 56, 91, 133, 182, 196 and 210
Food consumption:	Pretest and weekly
Body weights:	Pretest and weekly
Ophthalmoscopy:	Pretest, days 28, 91, 182 and 210 (recovery)
Electrocardiogram:	Pretest, days 28, 91, 182 and 210 (recovery)
Hematology:	Pretest, days 28, 91, 182 and 210 (recovery)
Clinical chemistry:	Pretest, days 28, 91, 182 and 210 (recovery)
Urinalysis:	Days 182 and 210 (recovery)
Organ weights:	Days 182 and 210 (recovery)
Histopathology:	Days 182 and 210 (recovery)
Toxicokinetics:	Days 0/1 and 181/182

Mortality (Daily)- One female (No.654) in the high dose (1538 mg/day) recovery group was found dead on day 131. This animal was subjected to a complete gross necropsy and the complete list of tissues was collected for histopathological examination. During necropsy, a hairless area at the base of tail, red diffuse areas in the thymus and some hemorrhagic infiltration in the vaginal wall were noted. The content of the stomach appeared dry and the mucous membrane was partly detached which according to the sponsor, was possibly due to autolytic changes. Histopathological examination did not reveal any treatment-related microscopic findings. However in most cases the

Sponsor stated that the tissue was slightly autolytic and adequate only for superficial histologic examination. The death was not considered test article-related.

Reviewer Note: Regarding toxicology study #20-3-0120-12, the toxicology tabulated summary on page 23, states: "One female of the high dose recovery group (No.652) was found dead at day 131." However the actual study report, on page 48, states that female No. 654 was found dead on Day 131. The sponsor will be asked to clarify and correct this discrepancy.

Clinical Signs (Pretest and days 7, 14, 28, 56, 91, 133, 182, 196 and 210 [recovery])- No test article-related effects were observed.

Food consumption (Pretest and weekly)- No test article-related effects were observed.

Body Weights (Pretest weekly)- No test article-related effects were observed.

Ophthalmoscopy (Pretest, days 28, 91, 182 and 210 [recovery])- Rare and sporadic ophthalmic observations (hyperemia, dry/closed eyelid) were likely vehicle or procedure related as they occurred at the same incidence in control and test-article treated animals. Pupillary reflex appeared reduced in one 526 mg/day female on day 28.

Electrocardiogram (Pretest, days 28, 91, 182 and 210 [recovery])- The P wave was significantly longer (+21%; $p \leq 0.05$) in the males dosed with 1026 mg/day than in the corresponding controls on day 28. This was the only ECG finding. This reviewer considered this finding not treatment related as there was no dose response, the increase was transient and the finding was only observed in one animal.

Hematology (Pretest, days 28, 91, 182 and 210 [recovery])- On day 91, activated partial thromboplastin time (aPTT) was reduced in females dose with 1026 mg/day (-24%; $p<0.05$), as well as in females dose with 1538 mg/day (-32%; $p<0.001$; main study and recovery animals combined). On day 182, activated prothrombin time was decreased in females dosed with 513 mg/day and 1026 mg/day (-24% and -25.5%, respectively, $p<0.05$). aPTT was also reduced in females treated with 1538 mg/day (-13.5%) but this was not statistically significant. This effect was not observed after a recovery period of 4 weeks.

Percent Change in Hematology Parameters (Days 91, 180 and 210 [recovery])

	Dose (mg/day)			
Parameter	**513**	**1026**	**1538**	**1538 +recovery (Day 210)**
aPTT (m/f) Day 91	-/-	-/-24	-/-32**[a]	N/A
aPTT (m/f) Day 180	-/-24*	-/-25.5*	-/-13.4	N/A
aPTT (m/f) M7	N/A	N/A	N/A	-/-

Day 210				

*P<0.05; *P<0.001; [a]main and recovery animals combined; N/A- not applicable

Clinical Chemistry (Pretest, days 28, 91, 182 and 210 [recovery])- In recovery males dosed with 1538 mg/day, glutamate dehydrogenase was increased by 100% ($p<0.05$) while, triglycerides were increased by 74% ($p<0.05$).

Urinalysis (At necropsy)- No test article-related effects were observed.

Organ weights (At necropsy)- No test article-related effects were observed.

Histopathology

Adequate Battery- Yes

Signed Pathology Report – Yes

Peer Review- No

Macroscopic Findings: Red spots on the lung, were observed in one male dosed with 513 mg/day and one female dose with 1538 mg/day. Enlarged/dark adrenals were observed in one male dosed with 1538 mg/day. Spleen like tissue was observed in the pancreas in one male dosed with 1026 mg/day. Small gall bladder was observed in one female dosed with 1538 mg/day. Mesenteric lymph nodes appeared dark in one female dose with 1026 mg/day. These effects were not observed in recovery animals and their significance was unclear without a histopathological correlate.

Microscopic Findings:
Non-recoverable effects-
Multinucleated giant cells were observed at ≥513 mg/day in the epididymis in one male and the effect was also observed after recovery in one male. Mononuclear cell infiltrate of the eyes was observed at a higher incidence in test-article treated animals than controls. Erythrophagocytosis was observed at all doses of F4H5. Glandular dilation of the mammary gland was observed in females at all doses. Rathke's pouch dilation was observed at 1538 mg/day (Table 13).

Recoverable effects-
Mononuclear cell infiltrate was observed in the lacrimal glands at 513 mg/day F4H5. Hypertrophy of the liver was observed in females at ≥513 mg/day. Mononuclear cell infiltrate in the lungs was observed in both males and females dosed with F4H5. Hemorrhage/blood resorption were observed in males and females dosed with ≥1026 mg/day. Tubular atrophy of the testes was observed at ≥513 mg/day (Table 13).

This reviewer noted these findings but did not consider them test article-related.

Table 13: Microscopic Findings:

Tissue/finding	# examined & severity	Male						Female					
Dose (mg/day)		0	0+r*	513	1026	1538	1538 +r*	0	0+r*	513	1026	1538	1538+r*
Epididymides	# examined	6	4	6	6	6	4	-	-	-	-	-	-
Multinucleated giant cells	Grade 1	0	0	1	0	0	1	-	-	-	-	-	-
Eyes	# examined	6	4	6	6	6	4	6	4	6	6	6	4
Mononuclear cell infiltrate	Grade 1	0	0	2	0	2	2	0	1	2	3	1	0
Lacrimal glands	#examined	6	4	6	6	6	4	6	4	6	6	6	3
Mononuclear cell infiltrate	Grade 1	0	0	3	0	2	0	0	0	0	0	2	0
Liver	# examined	6	4	6	6	6	4	6	4	6	6	6	4
Hypertrophy	Grade 1	0	0	0	0	0	0	0	0	1	1	0	0
Lungs	# examined	6	4	6	6	6	4	6	4	6	6	6	4
Mononuclear cell infiltrate	Grade 1	0	0	1	1	0	0	1	0	3	0	2	0
	Grade 2	0	0	0	0	0	0	0	0	0	1	0	0
Mesentery lymph node	# examined	6	4	6	6	6	4	6	4	6	6	6	4
Hemorrhage/blood resorption	Grade 1	0	0	0	0	1	0	0	0	0	1	0	0
Erythrophagocytosis	Grade 1	0	0	2	2	1	2	1	2	4	2	0	1
	Grade 2	0	0	1	0	0	0	0	0	0	0	0	0
Mammary gland	# examined	6	4	6	6	6	3	6	4	6	6	6	4
Glandular dilation	Grade 1	0	0	0	0	0	0	0	1	0	0	0	0
	Grade 2	0	0	0	0	0	0	0	0	2	1	1	2
	Grade 3	0	0	0	0	0	0	0	1	0	0	0	0
Pituitary gland	# examined	6	2	6	6	6	4	6	4	6	6	6	4
Rathke's pouch dilation	Grade 1	0	0	0	0	0	0	0	0	0	0	2	0
	Grade 2	0	0	0	0	0	1	0	1	0	0	0	0
Testes	# examined	6	4	6	6	6	4	-	-	-	-	-	-
Tubular atrophy	Grade 1	0	0	1	0	1	0	-	-	-	-	-	-

+r= recovery; - = N/A

Toxicokinetics (Days 0/1 and 181/182)- Toxicokinetic investigations revealed a generally low systemic exposure to F4H5 at all dose levels, although inter-individual variability was generally high at all dose levels and over the duration of treatment (Table 14). Overall, the systemic exposure remained nearly constant during the 6-months treatment period. In male and female rabbits, C_{max} was attained very early at the first measured time point (0.13 h; same as in 28 day study) at the beginning as well as at the end of the treatment period. Half-lives ranged between 5.5 and 21.5 hours on Day 0 and 8.1 to 21.5 hours on day 181. Absolute AUC- as well as C_{max} values on treatment day 0 and day 181 were also similar in all dose groups (except females dosed with 1026 mg F4H5 as measured on Day 0), indicating a similar systemic exposure irrespective of the applied dose. It's unclear why such high exposure was observed in females dosed with 1026 mg/day on Day 0. Female AUC_{last} values were in the expected range on day 181. Exposure after each administration was similar in all dose groups, although the animals of the low, mid and high dose groups received one, two or three of such administrations per day, respectively.

Table 14: Toxicokinetic parameters of F4H5 after subconjunctival administration to rabbits for six months daily.

Day	Parameter	Unit:	513 mg/d	1026 mg/d	1538 mg/d
Males (Mean Values)					
Day 0	AUC_{last}	h*ng/mL	347	513	389
	AUC_{last}/D	h*ng/mL/mg	0.6	0.5	0.2
	T_{max}	h	0.13	0.13	0.13
	C_{max}	ng/mL	131	251	607
	C_{max}/D	ng/mL/mg	0.2	0.2	0.3
	$T_{1/2}$	H	13.0	21.5	5.5
	Lz	1/h	0.05	0.03	0.13
Day 181	AUC_{last}	h*ng/mL	426	451	633
	AUC_{last}/D	h*ng/mL/mg	0.8	0.4	0.4
	T_{max}	h	0.13	0.13	0.13
	C_{max}	ng/mL	177	137	190
	C_{max}/D	ng/mL/mg	0.3	0.1	0.1
	$T_{1/2}$	H	21.1	11.3	11.1
	Lz	1/h	0.03	0.04	0.06
Females (Mean Values)					
Day 0	AUC_{last}	h*ng/mL	625	4554	468
	AUC_{last}/D	h*ng/mL/mg	1.2	4.4	0.3
	T_{max}	h	0.13	0.13	0.13
	C_{max}	ng/mL	381	251	356
	C_{max}/D	ng/mL/mg	0.7	0.2	0.2
	$T_{1/2}$	H	15.4	21.5	17.2
	Lz	1/h	0.05	0.03	0.04
Day 181	AUC_{last}	h*ng/mL	522	362	250
	AUC_{last}/D	h*ng/mL/mg	1.0	0.5	0.3
	T_{max}	h	0.13	0.13	0.13
	C_{max}	ng/mL	244	228	180
	C_{max}/D	ng/mL/mg	0.4	0.2	0.1
	$T_{1/2}$	H	8.1	9.7	9.2
	Lz	1/h	0.09	0.07	0.08

Excerpted from the sponsor's study report, page 667 and 668

6.3 Tolerance

Study title: Local Tolerance Study of Cyclosporin A in F4H5/Ethanol in New Zealand White Rabbits after repeated administration into the conjunctival sac for 28 days (including Pharmacokinetic Investigations)

Study no.:	20-3-0116-12
Study report location:	EDR Module 4.2.3.6
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 22, 2012
GLP compliance:	Principles of Good Laboratory Practice
	(b) (4)
QA statement:	Yes
Drug, lot #, and % purity:	Cyclosporin A in F4H5/Ethanol, Batch No. Cyclo25062012 and 100.2% cyclosporin, 0.47% (m/m) ethanol, (b) (4) F4H5

Key Study Findings

- Cyclosporin A in F4H5/Ethanol or RESTASIS® were administered in the conjunctival sac at doses of 100µL/eye/day (0.5mg CsA/eye/day) on Days 0-27 to male and female rabbits. Cyclosporin A in F4H5/Ethanol treated animals received (b) (4) F4H5/eye/day.
- No ocular effects were associated with ocular administration of Cyclosporin A in F4H5/Ethanol. No systemic effects of the test article were noted clinically or on gross examination.
- Blood levels of CsA could not be detected in this study.
- Higher concentrations of CsA were found within 24hr after administration in lacrimal gland (12x) and cornea (2x) in the Cyclosporin A in F4H5/Ethanol formulation compared to RESTASIS®.
- On Day 27, CsA concentrations remained higher in cornea in the CsA(F4H5) group (2x) compared to RESTASIS® group, with no observed accumulation. Lacrimal gland CsA levels were approximately equivalent in the CsA(F4H5) and RESTASIS® groups on Day 27.

Methods:

Doses: Groups I - isotonic saline solution, 50µL/eye, BID, OU
Group II - Cyclosporin A in F4H5, 50µL/eye, BID, OU (0.5mg CsA/eye/day and (b) (4) F4H5/eye/day)
Group III - RESTASIS®, 50µL/eye, BID, OU (0.5mg CsA/eye/day)
Groups IV - isotonic saline solution, 50µL/eye, BID, OU
Group V - Cyclosporin A in F4H5, 50uL/eye BID, OU (0.5mg CsA/eye/day and (b) (4) F4H5/eye/day)
Group VI - RESTASIS®, 50µL/eye, BID (0.5mg CsA/eye/day)
(Italicized groups were included for PK analysis)

Frequency of dosing: BID for 28 days (Groups I-III)
BID for 1 day (Groups IV-VI; PK group)

Route of administration: Topical ocular, into the conjunctival sac

Dose volume: 0.05 mL/eye, used a disposable pipette

Formulation/Vehicle: The study report states the test article is (b) (4) (b) (4) of *Cyclosporin A in F4H5/Ethanol and lists the active and inactive ingredients, as follows:*
Cyclosporine A (b) (4)
Inactive ingredients: F4H5 (perfluorobutylpentane) , Ethanol (b) (4) M%)

(b) (4)

Species/Strain: SPF rabbits [Crl:KBL (NZW)]

Number/Sex/Group: 5M, 5F (groups I - III)
3M, 3F (groups IV - VI)

Age: 2 - 3 months (groups I - III)
3 - 4 months (groups IV - VI)

Weight: 2.5 - 3.2 kg (groups I - III)
3.0 - 3.8 kg (groups IV - VI)

Satellite groups: Groups IV-VI are PK groups = 3/sex/group

Unique study design: Ocular PK included in this study design

Deviation from study protocol: No deviations that affect interpretation of the study.

Group	No. of Animals	Treatment			Necropsy on Day	
		Article	Days	Dose[1]	0	27
I *Negative Control*	5 m 5 f	*Isotonic Saline Solution*	0 - 27	2 x 50 µl/eye		X
II *Test*	5 m 5 f	*Cyclosporin A in F4H5/Ethanol*	0 - 27	2 x 50 µl/eye		X
III *Positive Control*	5 m 5 f	*RESTASIS®*	0 - 27	2 x 50 µl/eye		X
IV *Negative Control*	3 m 3 f	*Isotonic Saline Solution*	0	2 x 50 µl/eye	X	
V *Test*	3 m 3 f	*Cyclosporin A in F4H5/Ethanol*	0	2 x 50 µl/eye	X	
VI *Positive Control*	3 m 3 f	*RESTASIS®*	0	2 x 50 µl/eye	X	

[1] administered 2x per day at intervals of approximately 6 h

Excerpted from Sponsor's study Report No. 20-3-0116-12, p29

Observations and Results

Day	Treatment	Body Weight	Special Ophthalmological Examinations	Macroscopic Ocular Reactions	Blood Collection (Pharmacokinetics)[1]	Necropsy
-1			I-III			
0	I-III (2x) IV-VI (2x)	I-III IV-VI	IV-VI[3]		I (1x) II-III (7x)	IV-VI[2]
1	I-III (2x)			I-III	II-III (1x)	
2 - 6	I-III (2x)					
7	I-III (2x)	I-III	I-III			
8 - 13	I-III (2x)					
14	I-III (2x)	I-III	I-III			
15 - 20	I-III (2x)					
21	I-III (2x)	I-III				
22 - 26	I-III (2x)				I (1x) II-III (7x)	
27	I-III (2x)	I-III	I-III	I-III	II-III (1x)	I-III[2]

[1] from 3 males and 3 females of groups I - III

[2] including blood collection prior to necropsy (pharmacokinetics)

[3] prior to the 1st treatment on this day

Note: General health observations were conducted regularly throughout the entire study period (see III. 3.6).

Excerpted from Sponsor's study Report No. 20-3-0116-12, p30

Table 1 - Proposed observations and times

Mortality:	Daily
Clinical signs:	Daily
Body weights:	Days 0, 7, 14, 21 and 27
Food consumption:	Daily
Ophthalmological Examinations	See chart above
Macroscopic Ocular Observations:	Days 1 and 27 prior, included cornea, corneal reflex, iris, conjunctivae and chemosis
Gross pathology:	Animals were necropsied on Day 27 (main study) or Day 0 (ocular PK group). A general post mortem exam was performed, including eyes and surrounding tissues. Organs with treatment related changes preserved.
Ocular	Animals were necropsied on Day 27 (main study) and left eye and optic

Histopathology:	nerve preserved for histopathology
Ocular Pharmacokinetics:	On Day 27 (main study) or Day 0 (ocular PK group) a sample of aqueous humor was collected from the right eye, then the cornea, conjunctiva (upper eyelid: subsample, size approximately 1 x 0.3 cm) and lacrimal gland were collected, weighed and frozen for analysis.
Toxicokinetics:	Blood samples were taken from the jugular vein as follows: *Group I*: rabbit nos. xx1, xx3, xx5: 30 min after the last treatment on days 0 and 26 Groups II - III: Rabbit nos. xx1, xx3, xx5: 8, 15, 30 min and 1, 2, 4, 8 and 18 h after the last treatment on days 0 and 26 All rabbits: 30 min after the last treatment on day 27 (i.e. prior to sacrifice for necropsy) Groups IV - VI: All rabbits: 30 min after the last treatment on day

The following were not assessed in this study: ECG, **hematology, clinical chemistry, coagulation, urinalysis and organ weights.**

Mortality, Clinical Signs, Body Weight and Food Consumption

No unscheduled deaths were deemed test article related. No reported clinical signs or variations in body weight and food consumption were test article related.

After the first blood collection on Day 0 a single rabbit (no. 351) was found recumbent, with a reduced respiratory rate and swelling in the larynx area and was found dead that afternoon. Necropsy revealed an extensive hematoma and pale skeletal muscle attributed to traumatic blood collection.

Ophthalmological Examinations

No test article related effects were noted.

Gross Pathology

No test article related effects were noted.

Histopathology

Pathology report indicates the following tissues of the left eye of all animals in group I – III were examined: cornea, uvea, sclera, eye lens, retina, anterior chamber, posterior chamber vitreous body, optic nerve, conjunctiva and periocular tissues.

Adequate Battery – A microscopic evaluation of the nasal mucosa was not included.

Signed Pathology Report – Yes

Peer Review- No

Histological Findings: No test article related effects were noted.

Ocular Pharmacokinetics and Toxicokinetics

As shown in the chart below, on Day 0, the average CsA concentrations in the lacrimal gland and cornea of the CsA(F4H5) group were approximately 12 and 2 times higher, respectively, compared to the RESTASIS® group. At Day 27 the CsA concentrations in the lacrimal gland were similar in both groups, while CsA concentrations in the cornea were approximately 2 times higher in the CsA(F4H5) group compared to the RESTASIS® group. CsA appeared to distribute faster to the lacrimal gland and cornea following administration of the CsA(F4H5) formulation compared to RESTASIS®. On Day 27, CsA concentrations in the cornea remain higher in the CsA(F4H5) group compared to RESTASIS® group, with no apparent accumulation between Day 0 and Day 27.

day 0	**lacrimal gland**		**conjunctiva**		**cornea**		**blood**	
	AV	SD	AV	SD	AV	SD	AV	SD
4 saline	<LLOQ	-	<LLOQ*	-	<LLOQ	-	<LLOQ	-
5 CsA (F4H5)	169.4	150.0	412.0	91.0	1326.2	235.8	<LLOQ	-
6 Restasis®	14.0	8.3	575.8	460.9	633.7	257.8	<LLOQ	-
day 27	AV	SD	AV	SD	AV	SD	AV	SD
1 saline	<LLOQ	-	8.9	9.2	<LLOQ	-	<LLOQ	-
2 CsA (F4H5)	143.9	136.0	751.7	291.6	3462.1	840.0	<LLOQ	-
3 Restasis®	167.9	229.8	684.1	322.0	1451.3	408.7	<LLOQ	-
AV = average; SD = standard deviation; LLOQ = lowest limit of detection								

*one out of six values was 3.6 ng/g

Excerpted from Sponsor's study Report No. 20-3-0116-12, p175

In the blood, cyclosporine values could not be determined in this study. As indicated in the chart above, all measured values were below the lowest limit of quantification (LLOQ) of the method (LC/MS).

Stability and Dosing Solution Analysis

Samples obtained for concentration verification analysis were within the acceptable limits of ±10% error. The dosing solution is stable at room temperature (15 - 25° C). Studies were completed before the stability expiration date of December 25, 2012.

7 Genetic Toxicology

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

Study title: Bacterial Reverse Mutation Test with F4H5

Study no.:	16G10022
Study report location:	\\CDSESUB1\EVSPROD\nda217469\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\16g10022\16g10022-pre-clinical-study-report.pdf
Conducting laboratory and location:	(b) (4)
Date of study initiation:	5/19/2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	F4H5 (Perfluorobutylpentane), 90051, 99.8%

Key Study Findings

- F4H5 (perfluorobutylpentane) did not cause a positive increase in the mean number of revertants per plate with any tester strains either in the presence or absence of microsomal enzymes (S9) in this assay, which was modified for testing vapors.
- The results indicate that F4H5 (perfluorobutylpentane) is not genotoxic under the conditions of this study.

Methods

Strains:	*Salmonella typhimurium*: TA 98, TA 100, TA 1535, TA 1537; *Escherichia coli*: E. coli WP2 uvra (pkm101)
Concentrations in definitive study:	For the plate incorporation method, concentrations were 0.014%; 0.038%; 0.1%; 0.3%; 0.7% F4H5 (with and without S9-mix) dose/plate determined at the beginning and the end of the exposure period by gas chromatography - flame ionization detector (GC/FID) method
Basis of concentration selection:	The prevailing vapor pressure of F4H5 under standard atmosphere was the criterion to determine the highest concentration of the test item. At 0.7% F4H5 was not toxic to any tester strains in the presence or absence of metabolic activation.

Positive control:

Strain	TA 98	TA 100	WP2 uvrA (pkM101)	TA 1535	TA 1537
Without metabolic activation; (concentration)	2-Nitrofluorene in DMSO (5 µg/plate)	2-Nitrofluorene in DMSO (5 µg/plate)	Methyl methane-sulfonate in DMSO (2 µl/plate)	Sodium azide in H_20 (1µg/plate)	9-Aminoacridine in DMSO (50 µg/plate)
With metabolic activation; (concentration)	2-Amino-anthracene in DMSO (1 µg/plate)	2-Amino-anthracene in DMSO (1 µg/plate)	2-Aminoanthracene in DMSO (1 µg/plate)	2-Amino-anthracene in DMSO (1 µg/plate)	2-Amino-anthracene in DMSO (1 µg/plate)

Formulation/Vehicle: DMSO or H2O, Ambient Air

Incubation & sampling time: Due to the volatility of the test compound, the assay was modified for testing vapors. Three glass jars, containing 12 glass petri dishes each, were hermetically sealed, a slight vacuum was created and a liquid aliquot of the test article was injected into the vessels. After vaporization and mixing of the test compound standard pressure conditions were restored. Tester strains were incubated at 37°C and exposed under the above conditions to the test article for 48 hr.

Study Validity

- Triplicate cultures (within-assay)
- Duplicate independent assays
- Revertant colonies were counted by automated colony counter.

Criteria for a positive response were provided in the study report:

- For tester strains, TA98, TA100 andWP2uvrA, the test article was considered positive if it produced at least a 2-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase in the mean number of revertants per plate should be accompanied by a dose response to increasing concentrations of the test article.
- For tester strains, TA1535 and TA1537, the test article was considered positive if it produced at least a 3-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase in the mean number of revertants per plate should be accompanied by a dose response to increasing concentrations of the test article.

Results

- There was no evidence of dose-related cytotoxicity in any of the strains tested, in either the presence or absence of S9.
- All data were considered acceptable, and no increase in the mean number of revertants per plate was observed with any of the tester strains, in either the presence or absence of S9 mix.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: In Vitro Mammalian Chromosome Aberration Test (Human Lymphocytes) with F4H5

Study no.:	17G10021
Study report location:	\\CDSESUB1\EVSPROD\nda217469\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\17g10021\17g10021-pre-clinical-study-report.pdf
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/7/2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	F4H5 (Perfluorobutylpentane), Batch No. 90051, and 99.8% (b) (4) - (b) (4)

Key Study Findings

Methods

Cell line:	Phytohemagglutinine (PHA)-stimulated human lymphocytes human peripheral lymphocytes from a healthy male donor
Concentrations in definitive study:	4 h without S9-mix: 0, 600, 1200 and 1800 µg/ml 4 h with S9-mix: 0, 50, 200, 600 and 1800 µg/ml 24 h without S9-mix: 0, 2.5, 7.5, 20 and 40 µg/ml
Basis of concentration selection:	A preliminary dose range-finding study was conducted using a range of test concentrations from 125 µg/mL to 5000 µg/mL. Doses were determined based on toxicity (mitotic indices (MI) = number of metaphases/number of cells counted x 100). Toxicity (MI) below 50% (in percent of negative control) were observed at doses ≥ 1000 µg/mL in cultures without S9-mix, while no toxicity was noted at any dose with S9-mix after 4 h continuous exposure. During the 24 h continuous exposure conditions, the MI in the presence of drug at all doses tests was ≤ 35% of the negative control. Based on these pre-test results 600 µg/mL was chosen as the maximum concentration for long term (24 h) definitive studies, however there was severe cytotoxicity at this dose that prevented chromosome analysis, so in maximum concentration was reduced to 40 µg/mL in subsequent assays.

Negative control:	4 h studies: Dulbecco's-minimum essential medium (DMEM) with high glucose, GlutaMaxTM, sodium pyruvate, and 10 (without S9-mix) or 2% bovine serum (with S9- mix) 24 h studies: chromosome medium B (without S9-mix)
Positive control:	Ethyl methanesulfonate (EMS) at final concentrations of 1000 µg/mL – without S9-mix Cyclophosphamide monohydrate (CP) at final concentrations of 5.0 and 7.5 µg/mL – with S9-mix
Formulation/Vehicle:	Emulsions of F4H5 prepared using (b) (4)
Incubation & sampling time:	The cells were incubated with or without metabolic activation in a cell culture incubator at 37°C, 5% CO_2, 90% humidity. Duplicate cultures were used for all samples.

Exposure conditions and sampling time

Treatment Condition	Treatment Time	Drug Concentration
Non activated (-S9)	4 h	0, 600, 1200 and 1800 µg/ml
	24 h	0, 2.5, 7.5, 20 and 40 µg/ml
Activated (+S9)	4 h	0, 2.5, 7.5, 20 and 40 µg/ml

Study Validity

- Duplicate cultures were used at each test article concentration, for the vehicle controls, and for the positive controls.
- Analysis of chromosomal aberration was performed on a minimum of 3 selected test concentrations (on the basis of mitotic suppression), and on concurrent negative and positive controls.
- One hundred acceptable diploid metaphase cells from each culture were evaluated for chromosome damage.
- Evaluation of a Positive Result:
  - Effect must be dose-related and reproducible in the treated groups.
  - At least one concentration must produce a statistically significant increase in aberrant cells .
  - An equivocal response to a test compound was indicated by a statistically significant increase in aberrant cells observed at only one treatment concentration.
  - Mitotic suppression should optimally be 50%, and not exceed 69%, compared to controls.

Results

- For the 4-hour non-activated group, the highest test article concentration tested (1800 µg/mL) reduced the mean mitotic index (MI) to 43% as compared to the vehicle control (set to 100%). The concentrations selected for analyses were 600, 1200, and 1800 µg/mL. The percentage of cells with structural aberrations

was not significantly different from controls at the any of the concentrations examined. The positive control, EMS (1000 µg/mL) and EMS (1000 µg/mL)+vehicle control, produced an increase in the percentage of cells with structural aberrations; 45% (including gaps) and 32.5% (excluding gaps) and 50% (including gaps) and 33.0% (excluding gaps), respectively.

- For the 4-hour activated group, the highest test article concentration tested (1800 µg/mL) reduced MI to 29% relative to controls. The concentrations selected for analysis were 50, 200, 600, and 1800 µg/mL. The percentage of cells with structural aberrations in the treated groups was not significantly different from controls at the any of the concentrations examined. The positive control, cyclophosphamide (7.5 µg/mL) and cyclophosphamide (5.0 µg/mL) + vehicle control increased the percentage of cells with structural aberrations, 45% (including gaps) and 20% (excluding gaps) and 41% (including gaps) and 19.5% (excluding gaps), respectively.
- For the 24-hour non-activated group, the highest test article concentration used (40 µg/mL) reduced MI to 33% relative to controls. The concentrations selected for analyses were 1.0, 2.5, 7.5, 20 and 40 µg/mL. The percentage of cells with structural aberrations was not significantly different from controls at the any of the concentrations examined. The positive control, cyclophosphamide (500 µg/mL) and cyclophosphamide (500 µg/mL) + vehicle control increased the percentage of cells with structural aberrations, 33% (including gaps) and 18% (excluding gaps) and 47.5% (including gaps) and 23.5% (excluding gaps), respectively.
- In conclusion, F4H5 was considered to be negative in the in vitro chromosome aberration assay under the conditions tested.

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

Study title: Mammalian Erythrocyte Micronucleus Test with F4H5

Study no:	17G11002
Study report location:	\\CDSESUB1\EVSPROD\nda217469\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\17g11002\17g11002-pre-clinical-study-report.pdf
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1/25/2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	F4H5 (Perfluorobutylpentane), Batch No. 90051, and 99.8% (b) (4)

Key Study Findings

- F4H5 was tested for genotoxic potential in an in vivo micronucleus (MN) test. A single dose was administered to male and female mice (6/F4H5 group/time point) at doses of 1000, 2000, and 3000 mg/kg. Bone marrow was collected at 24 hours post dose (positive control, 1000 and 2000 mg/kg) or both 24 and 48 hours post dose (negative control and 3000 mg/kg) for evaluation of the frequency of MN polychromatic erythrocytes (MNPCE) and the frequency of MN reticulocytes (MNRET).
- Administration of F4H5 to male and female mice at oral doses of 1000, 2000, or 3000 mg/kg/day produced no mortality, no clinical signs, no changes in body weight and no statistically significant or dose-related increases in the frequencies of MNPCE.
- F4H5 was judged to be negative in the micronucleus test incorporated in a single dose study with male and female mice.

Methods

Doses in definitive study:	In a single dose acute study with F4H5, 6 male and 6 female mice/group/time point received F4H5 at oral doses of 1000, 2000, and 3000 mg/kg. No vehicle or drug suspension was administered, as F4H5 is a liquid and was administered directly by oral gavage. No dose volume was provided. Mice were sacrificed at 24 hours post dose (positive control, 1000 and 2000 mg/kg) or both 24 and 48 hours post dose (negative control and 3000 mg/kg) or for bone marrow sampling. A positive control group (5M, 5F), cyclophosphamide monohydrate, PO, 60 mg/kg, dissolved in tap water, dose volume of 10 ml/kg was used. Negative control (5M, 5F) was sham treatment. Bone marrow cells collected from animals treated with a single dose of F4H5 were stained with May-Grünwald- and Giemsa solution. The incidence of micronuclei was determined by evaluating 2000 polychromatic erythrocytes (PCEs) per animal were manually evaluated. Further, 200 RBCs per animal were counted for determination of the ratio of PCE to all erythrocytes (normal chromatic erythrocytes, NCE). The PCE to NCE served as an index of bone marrow toxicity.

Frequency of dosing:	Single administration
Route of administration:	Oral gavage
Dose volume:	10mL/kg
Formulation/Vehicle:	None.
Species/Strain:	NMRI mice obtained from (b) (4) (b) (4) At delivery, animals were approximately 8 weeks old. Body weight ranges at the start of treatment were 46.87 to 41.71 g for male rats and 29.3 to 34.97 g for female rats.
Number/Sex/Group:	6/sex/group
Satellite groups:	None.
Basis of dose selection:	This study did not include a pre-test on toxicity, but referenced two previous studies; (b) (4) studies No. 02N10515 and 17G10020 report and report findings as follows "*both male and female animals survived single oral doses of up to 4000 mg F4H5/kg without marked signs of acute toxicity without marked signs of acute toxicity.*"
Negative control:	Control animals received sham treatment.
Positive control:	A positive control group (5M, 5F), received cyclophosphamide monohydrate, PO by oral gavage, 60 mg/kg, dissolved in tap water, dose volume of 10 ml/kg was used.

Evaluation Criteria for a Positive Response:
Providing that all acceptability criteria are fulfilled, a test chemical is considered clearly positive if:

a) At least one of the treatment groups exhibits a statistically significant increase in the frequency of micronucleated immature erythrocytes compared with the concurrent negative control,
b) This increase is dose-related at least at one sampling time when evaluated with an appropriate trend test, and
c) Any of these results are outside the distribution of the historical negative control data (e.g. Poisson-based 95% control limits).

Study Validity

In the single dose study, male and female mice received F4H5 at oral doses of 1000, 2000, and 3000 mg/kg. No mortality, signs of acute toxicity or reduction in body weight was attributed to treatment.

Peripheral blood concentrations of F4H5 after dosing were not evaluated in this study. In female mice, F4H5 induced a statistically significant increase in PCE:NCE (blood formation in bone marrow) compared to negative controls at 48 hrs after application. In addition, in (b) (4) Study NO. 02N10518 (non-GLP) F4H5 was detectable at

30 and 60 minutes following oral administration of 2000 mg/kg in mice [Crl:DC1 (ICR), 12 weeks old] mice as seen in the table below.

Blood sampling [time after application]	Animal No.	F4H5 [ng/ml blood]	Mean ± SD [ng F4H5/ml blood]
30 min	1	131.1	249.1 ± 104.11
	2	288.2	
	3	328.0	
60 min	4	331.5	231.2 ± 103.70
	5	124.4	
	6	237.6	

SD: Standard deviation

Results

- F4H5 at single oral doses of 1000, 2000, and 3000 mg/kg produced no statistically significant or dose-related increases in the frequency of MN reticulocytes at 24 and 48 hrs after applications in male or female mice compared to the negative controls. In addition, observed incidences of micronuclei were within the laboratory historical control range.
- F4H5 was judged to be negative in the micronucleus test in a single dose study with male and female mice.

8 Carcinogenicity

- Carcinogenicity studies were conducted for the Listed Drug, Cyclosporine A, under NDA 050573, referenced in this 505(b)(2) NDA application. All statements in the labeling are based upon these findings with adjustments made for dose margins based on the clinical dose proposed in this application.

9 Reproductive and Developmental Toxicology

- Reproductive and developmental toxicology studies were conducted for the Listed Drug, Cyclosporine A, under NDA 050573, referenced in this 505(b)(2) NDA application. All statements in the labeling are based upon these findings with adjustments made for dose margins based on the clinical dose proposed in this application.

10 Special Toxicology Studies

Study title: Compounds F4H5 and F6H8. Evaluation of Tear Film Changes Following Multiple Daily Instillation in Albino Rabbits

Study no.:	N38D17113
Study report location:	EDR Module 4.2.3.7.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 16, 2013
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	F4H5 (perfluorobutylpentane), Reference/Batch No. 120075 P1, 99.86%

Key Study Findings

- F4H5 (perfluorobutylpentane) was administered topically in the right eye at doses of 120µL/eye/day (30µL, QID) on Days 1 – 7 to female rabbits. The left eye served as control.
- Under the study conditions, no significant differences were found in ocular examination, tear film break-up time (tBUT) or tear secretion measurement between F4H5 (perfluorobutylpentane) treated and control rabbit eyes.
- The concentration of perfluorobutylpentane is unclear.

Methods:

Doses:	30µL, OD, QID (120µL/day)
Frequency of dosing:	4x/day for 7 days
Route of administration:	Topical ocular, using provided dropper vial
Dose volume:	0.03mL/eye/dose (30µL/dose given in 3 x 10µL)
Treatment groups:	Group 1 - F4H5 Group 2 - F6H8 Group 3 - Vismed® multi
Species/Strain:	Rabbits/New Zealand White (albino)
Number/Sex/Group:	8F/group
Age:	8 - 12 weeks at the starting date
Weight:	2.0 – 2.5 kg (on ordering)
Deviation from study protocol:	None reported

Group No.	Treatment	Dose regimen from D1 to D7	Route of administration	Number of animals	Endpoints		
					tBUT	Schirmer	Ocular observations
1	Compound F4H5	Four times a day	Instillation (30 microL) in right eyes	8	Baseline, on D 1, 3 and 7		Baseline and twice a day from D1 to D7
2	Compound F6H8			8			
3	Vismed® multi			8			

Excerpted from Sponsor's study Report No. N38D17113, p8

Observations and Results

Mortality and Clinical Signs (observed daily)

No unscheduled deaths occurred and the general appearance and behavior of study animals were reported as normal.

Body Weight (once during pre-test period, then at end of study)

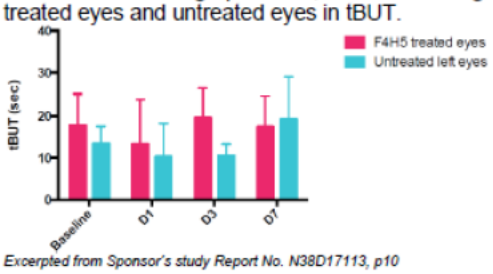
No test article related effects.

Ocular examination with an ophthalmoscope [pre-test baseline, 2x/day (before first dose and 5 minutes after last dose) on days 1 – 7; scored with Draize scale]

- Slight conjunctival redness (score 1 out of 4) was observed after the last dose on Day 2 for 1/8 treated with F4H5.
- These changes were mild and transient.

Tear film break-up time (tBUT) evaluation [pre-test baseline, days 1, 3 and 7 (just after first dose and 8-12 minutes after last dose of day; under slit-lamp the appearance of the first break in the tear film was recorded using a stop watch after a complete manual blink]

- As illustrated in the graph below, there was no significant difference between F4H5 treated eyes and untreated eyes in tBUT.



Excerpted from Sponsor's study Report No. N38D17113, p10

Tear secretion measurement [pre-test baseline, days 1, 3 and 7 (just after first dose and 13-17 minutes after last dose of day; Schirmer strips without anesthesia]

- No significant a difference in tBUT was found between F4H5 treated eyes and untreated eyes.

Reviewer Comments: The Sponsor will be asked to compare this formulation to that planned for the clinic.

Study title: Acute Dermal Irritation/Corrosion with Perfluorobutylpentane

Study no.:	053478
Study report location:	EDR Module 4.2.3.7.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 6, 2005, 2012
GLP compliance:	(b) (4)
QA statement:	Yes
Drug, lot #, and % purity:	Perfluorobutylpentane, Batch No. F4H5

Key Study Findings

- No local irritation (erythema and edema) was observed after 4 hrs of dermal contact with 0.5 mL of perfluorobutylpentane in the rabbit.
- The concentration of perfluorobutylpentane is unclear.

A patch with 0.5 mL of perfluorobutylpentane was applied to a 2.5 cm x 2.5 cm area (shaved ~24 hrs before) on the back (both sides of spinal cord) of three female rabbits (HsdIf:NZW, >2.0kg). Two control patches, moistened with isotonic saline NaCl 0.9% were also applied. The patches were held in place with surgical tape for 4 hours and then removed. Immediately and at 24, 48 and 72 hrs after bandage removal the skin was examined for erythema and edema. No erythema and edema were noted at any time point.

Reviewer Comments: The report does not specify the concentration of the perfluorobutylpentane*, nor does it comment on the size of the patches used. Therefore, the perfluorobutylpentane dermal dose cannot be calculated.*

Study title: Test for delayed-type hypersensitivity (Guinea Pig Maximisation Test) with Perfluorobutylpentane

Study no.:	053479
Study report location:	EDR Module 4.2.3.7.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 6, 2005, 2012
GLP compliance:	(b) (4)
QA statement:	Yes
Drug, lot #, and % purity:	Perfluorobutylpentane, Batch No. F4H5 250705, no purity data provided

Key Study Findings

- Perfluorobutylpentane did not evoke swelling or erythema following sensitization under the conditions of this study.
- The concentration of perfluorobutylpentane used in this study is unclear.

The test article (perfluorobutylpentane, Batch No. F4H5 250705, expiration 07/2009) was provided by the sponsor and used undiluted (100%) during the course of the study. This test article had the same Batch No. used in Study No. 053478, "*Acute Dermal Irritation/Corrosion with Perfluorobutylpentane*" reviewed above. No further information on the formulation is provided.

Two groups (test group - 10, control group - 5) of female guinea pigs (Hsd Poc: DH – Guinea pigs, 300-500g) were evaluated. The study involved the following phases:

1. Induction: first stage, intradermal injection, 0.1 mL (Day 1)

No. Animals	10	5
Injection	**Test Group**	**Control Group**
1	Freund's Adjuvant complete, 1 + 1 diluted with isotonic saline	Freund's Adjuvant complete, 1 + 1 diluted with isotonic saline
2	Perfluorobutylpentane	NaCl 0.9%
3	Perfluorobutylpentane 50% (v/v) in Freund's Adjuvant complete, 1 + 1 diluted with isotonic saline	NaCl 0.9% 50% (v/v) in Freund's Adjuvant complete, 1 + 1 diluted with isotonic saline

2. Induction: second stage, topical application (Days 6, 7)
   On Day 6 an "area" was closely clipped and painted with 0.5 mL of 10% sodium lauryl sulfate in vasoline. On day 7, a patch "fully loaded" with test item was applied and held on the skin for 48hr with an occlusive dressing. In addition a patch with NaCl 0.9% was applied to guinea pigs in the control group.

3. Challenge: topical application (Day 22)
   Patches were "loaded" with Perfluorobutylpentane (test group) or NaCl 0.9% (control group), applied to the closely-clipped flanks of the guinea pigs and held in contact for 24hr.

4. Observation: evaluation for skin reaction (Days 24, 26)
   The skin of the flank was evaluated for erythema and swelling the day following patch removal using the Magnusson and Kligman scale.

Reviewer Comments: The report does not specify the concentration of perfluorobutylpentane in the "test article" used in this study. Therefore, the precise exposure to perfluorobutylpentane is unclear.

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/

ERIN M RUHLAND
05/08/2023 01:32:12 PM

KIMBERLY P HATFIELD
05/08/2023 01:43:32 PM
I concur with the review and conclusions of Dr. Ruhland. Acting also on behalf of Dr. Lori Kotch.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

CLINICAL STUDIES

NDA/BLA #:	NDA 217469
Drug Name:	Vevye (Cyclosporine ophthalmic solution, 0.1%, Development Name: CyclASol)
Indication(s):	Topical treatment of the signs and symptoms of dry eye disease
Applicant:	Novaliq GmbH
Date(s):	Stamp Date: 08/08/2022 Review Date: 05/08/2023 PDUFA Date: 06/08/2023
Review Priority:	Standard
Biometrics Division:	Division of Biometrics IV
Statistical Reviewer:	Nam Hee Choi, Ph.D.
Concurring Reviewers:	Guoxing Soon, Ph.D., Team Leader
Medical Division:	Division of Ophthalmology
Clinical Team:	Rhea Lloyd, MD
Project Manager:	Crystal Bland

Keywords: Dry eye disease, signs and symptoms, superiority

Link to keywords:

http://intranetapps.fda.gov/scripts/ob_apps/ob/eWork/uploads/eWork/2009/Keywords-in-DFS.htm

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1 EXECUTIVE SUMMARY

This original NDA seeks approval of Vevye® (Cyclosporine ophthalmic solution 0.1%; product development name: CyclASol®) for the treatment of signs and symptoms of dry eye disease (DED). The NDA included one supportive Phase 2 study CYS-002, two pivotal studies CYS-003 (Phase 2b/3) and CYS-004 (Phase 3), and an open-label single-arm study CYS-005 which was an extension study to CYS-004 for evaluating long term safety and efficacy. For efficacy evaluation, the two pivotal studies CYS-003 and CYS-004 were used for the confirmatory evidence while CYS-002 was used as supportive evidence.

Both studies CYS-003 and CYS-004 were similar in design except for the study duration, the study size and the primary symptom endpoint. Both studies were randomized, double-masked, vehicle-controlled and multi-center clinical studies conducted in the US in subjects with dry eye disease (DED). Both studies consisted of two periods: a 14-day run-in period and then a treatment period. During the 14-day run-in period, subjects self-administered Systane Balance Lubricant Eye Drops bilaterally twice daily (BID). In Study CYS-003, 328 subjects were randomized in a 1:1 ratio to receive CyclASol 0.1% or the vehicle bilaterally BID for three months with the study visits on Days -14 (screening), 1 (baseline/randomization), 15, 29, 57, and 85. In Study CYS-004, 834 subjects were randomized in a 1:1 ratio to receive CyclASol 0.1% or the vehicle bilaterally BID for one month with the study visits on Days -14 (screening), 1 (baseline/randomization), 15, and 29.

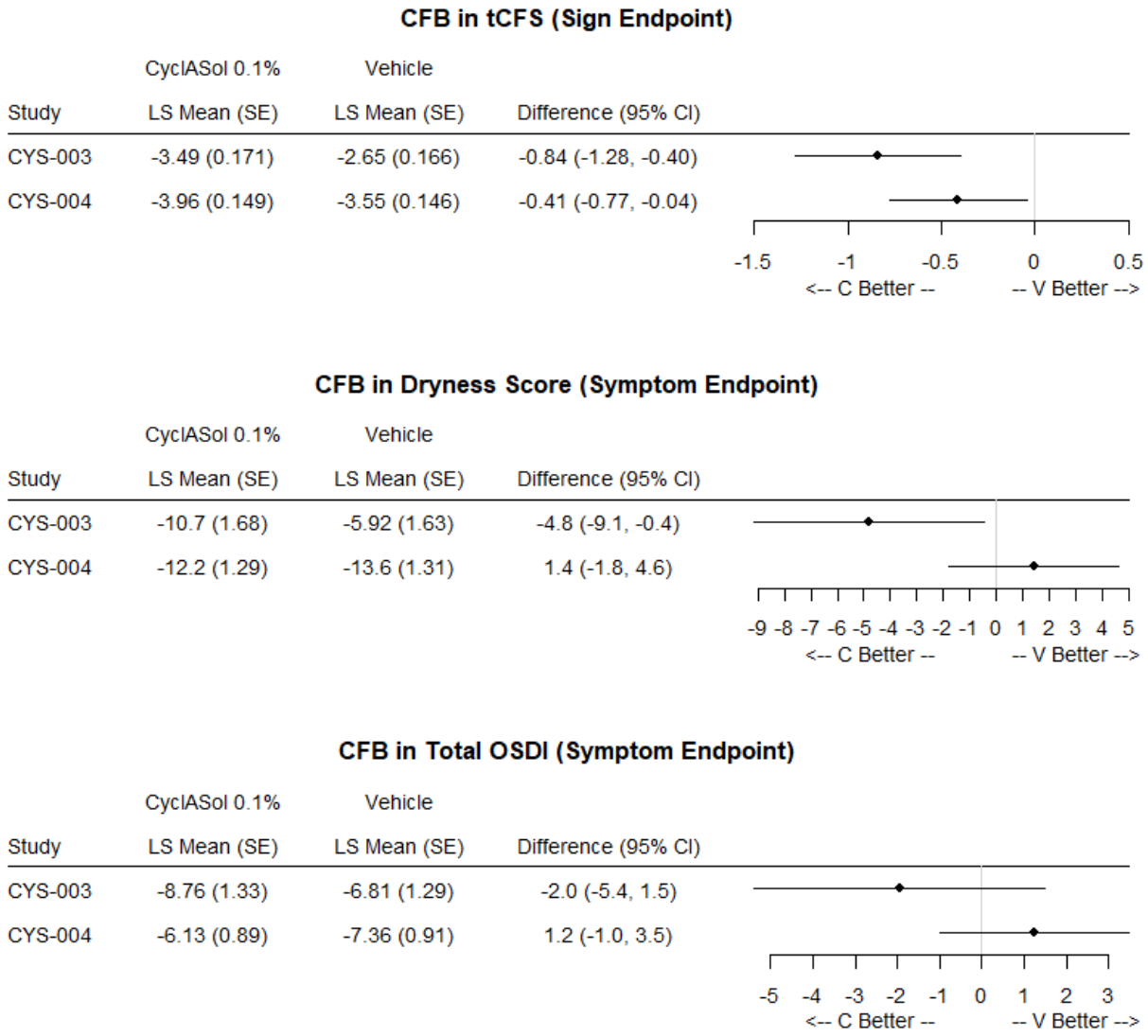
Both studies pre-specified one sign endpoint and one symptom endpoint as co-primary efficacy endpoints. The primary sign endpoint of both studies was change from baseline in total corneal fluorescein staining score (tCFS; National Eye Institute [NEI] scale) at Day 29. The primary symptom endpoints were different for the two studies: change from baseline in total Ocular Surface Disease Index (OSDI) score at Day 29 for Study CYS-003 and change from baseline in dryness score (Visual Analog Scale [VAS]) at Day 29 for Study CYS-004. Please see Table 2 for more details.

Figure 1 presents the summary of the primary efficacy results in the two pivotal studies CYS-003 and CYS-004. Both studies demonstrated superiority of CyclASol 0.1% to the vehicle regarding the primary sign endpoint, change from baseline in tCFS score at Day 29. Both studies failed to show superiority of CyclASol 0.1% to the vehicle regarding the pre-specified primary symptom endpoints (OSDI for CYS-003 and dryness score for CYS-004), respectively. Study CYS-003 showed superiority of CyclASol 0.1% to the vehicle regarding the change from baseline in dryness score, although it was not the pre-specified symptom endpoint for CYS-003. In Study CYS-004, the vehicle group showed numerically better reduction in dryness score than the CyclASol group.

Additionally, the Applicant conducted analysis based on the proportion of responders in Schirmer's tear test who had 10 mm or greater improvement from baseline. However, this analysis does not provide adequate evidence for superiority of CyclASol 0.1% to the vehicle, because it was not a pre-specified analysis, and the results are not robustly significant and lacks supportive evidence.

In conclusion, based on the collective evidence of the two pivotal studies, there is treatment effect of CyclASol 0.1% versus vehicle in terms of reducing total corneal fluorescein staining score (sign of dry eye disease), but it lacks evidence for the treatment effect of CyclASol 0.1% versus vehicle in terms of the co-primary endpoint for reducing dryness score or total Ocular Surface Disease Index score (symptoms of dry eye disease). Furthermore, the evidence on the Schirmer's tear test is post-hoc and marginal.

Figure 1 Summary of Efficacy Results in CYS-003 and CYS-004



The results are based on ANCOVA model with baseline value as covariate and site and treatment as factors.

2 INTRODUCTION

2.1 Overview

In this NDA, the Applicant seeks approval of Cyclosporine ophthalmic solution 0.1% (product development name: CyclASol®; proposed trade name: Vevye®) for the treatment of signs and symptoms of dry eye disease (DED). CyclASol contains the active pharmaceutical ingredient (API) cyclosporine at a concentration of 0.1% (w/v) in the excipient perfluorobutylpentane (often abbreviated F4H5). It is a clear and water-free solution.

The NDA included five clinical studies. Three of the studies, CYS-002, CYS-003 and CYS-004 were evaluated for efficacy. Study CYS-002 was a supportive Phase 2 study to explore the dose, endpoints and study population for the development of CyclASol. Study CYS-003 was a pivotal Phase 2b/3 study and Study CYS-004 was a pivotal Phase 3 study for evaluating efficacy and safety of CyclASol 0.1%. For safety evaluation, Study CYS-005 was included, which was an open label single arm extension study to Study CYS-004 for investigating the long-term safety and efficacy of CyclASol. The detailed descriptions of the study designs are presented in Table 1.

Table 1 List of All Studies Included in Analysis

Study	Phase and Design	Objectives	Treatment Duration	# of Subjects per Arm	Study Population
CYS-002 *(supportive)*	Phase 2 multicenter, randomized, double-masked, vehicle-controlled, with open-label comparator arm (Restasis)	Efficacy, safety and tolerability, PK	4 months	CyclASol 0.05%: 51 CyclASol 0.1%: 51 Vehicle: 52 Restasis: 53	207 subjects with DED
CYS-003 *(pivotal)*	Phase 2b/3 multicenter, randomized, double-masked, vehicle-controlled	Efficacy, safety and tolerability	3 months	CyclASol 0.1%: 162 Vehicle: 166	328 subjects with DED
CYS-004 *(pivotal)*	Phase 3 multicenter, randomized, double-masked, vehicle-controlled	Efficacy, safety and tolerability	1 month	CyclASol 0.1%: 423 Vehicle: 411	834 subjects with DED
CYS-005 *(supportive)*	Phase 3 multicenter, open-label, single arm extension study to CYS-004	Safety, tolerability and efficacy during long-term use	12 months	CyclASol 0.1%: 200	200 subjects with DED who had completed study CYS-004

2.2 Data Sources

The data sources for this review include the Applicant's clinical study reports, protocols, statistical analysis plans (SAPs), data sets, and responses to FDA's information requests (IRs). All data sources were electronically submitted and are located at:

- Study reports, protocols, and SAPs: \\CDSESUB1\evsprod\NDA217469\0001\m5\53-clin-stud-rep\535-rep-effic-safety-stud\ded\5351-stud-rep-contr
- Data sets for CYS-002: \\CDSESUB1\evsprod\NDA217469\0001\m5\datasets\cys-002

- Data sets for CYS-003: \\CDSESUB1\evsprod\NDA217469\0001\m5\datasets\cys-003
- Data sets for CYS-004: \\CDSESUB1\evsprod\NDA217469\0001\m5\datasets\cys-004
- IR responses: \\CDSESUB1\evsprod\NDA217469\0005\m1\us\111-information-amendment, \\CDSESUB1\evsprod\NDA217469\0006\m1\us\111-information-amendment

3 STATISTICAL EVALUATION

3.1 Data and Analysis Quality

Overall, the submitted data were of good quality with definitions provided for each variable. Results of the primary and secondary efficacy endpoints were verified by the statistical reviewer with minor data manipulation.

3.2 Evaluation of Efficacy

This section evaluates the efficacy results of the three studies, CYS-002, CYS-003 and CYS-004. Sections 3.2.1 and 3.2.2 summarize the study design, endpoints and statistical methods. Section 3.2.3 summarizes subject disposition as well as demographic and baseline characteristics. Section 3.2.4 discusses the primary analysis and supporting evidence.

3.2.1 Study Design and Endpoints

All three studies were randomized, double-masked, vehicle-controlled and multi-center clinical studies conducted in the US in subjects with dry eye disease (DED).

CYS-002 was a supportive Phase 2 study to explore the dose, endpoints, and study population for the development of CyclASol. Study CYS-002 contained four treatment arms: CyclASol 0.05%, CyclASol 0.1%, Vehicle and open-label Restasis. The study consisted of two periods: a 14-day run-in period and a 112-day treatment period. The scheduled study visits were: Visit 0 (Screening; Day -14), Visit 1 (Baseline/Randomization; Day 1), Visit 2 (Day 15), Visit 3 (Day 29), Visit 4 (Day 85), and Visit 5 (Study Exit; Day 113). The study was conducted at 4 investigative sites in the US.

CYS-003 was a pivotal Phase 2b/3 study to evaluate the efficacy, safety, and tolerability of CyclASol 0.1% compared to the vehicle in subjects with DED. The study consisted of two periods: a 14-day run-in period and an 84-day treatment period. The scheduled study visits were: Visit 0 (Screening; Day -14), Visit 1 (Baseline/Randomization; Day 1), Visit 2 (Day 15), Visit 3 (Day 29), Visit 4 (Day 57), and Visit 5 (Study Exit; Day 85). The study was conducted at 9 investigative sites in the US.

CYS-004 was a pivotal Phase 3 study to evaluate the efficacy, safety, and tolerability of CyclASol 0.1% compared to the vehicle in subjects with DED. The study consisted of two periods: a 14-day run-in period and a 29-day treatment period. The scheduled visits were: Visit 0 (Screening: Day -

14), Visit 1 (Baseline/Randomization; Day 1), Visit 2 (Day 15) and Visit 3 (Day 29). The study was conducted at 27 investigative sites in the US.

In all three studies, during the 14-day run-in period, subjects self-administered Systane Balance Lubricant Eye Drops bilaterally twice daily (BID), in the morning and in the evening at bedtime. During the treatment period, randomized subjects dosed the study drug bilaterally BID.

The detailed schedules of study visits and assessments of CYS-003 and CYS-004 are shown in Table 14 and Table 15 of Appendix 1.

The primary efficacy endpoints for the three studies are summarized in Table 2. The three studies pre-specified one sign endpoint and one symptom endpoint as primary efficacy endpoints.

In the Phase 2 study CYS-002, the primary sign endpoint was change from baseline total corneal fluorescein staining score (tCFS; National Eye Institute [NEI] scale) at Day 113 and the primary symptom endpoint was change from baseline in dryness score (Visual Analog Scale [VAS]) at Day 113.

The primary sign endpoint of the two pivotal studies CYS-003 and CYS-004 was change from baseline in total corneal fluorescein staining score (tCFS; National Eye Institute [NEI] scale) at Day 29. The primary symptom endpoints of the two studies were different. The primary symptom endpoint of Study CYS-003 was change from baseline in total Ocular Surface Disease Index (OSDI) score at Day 29, while the primary symptom endpoint of Study CYS-004 was change from baseline in dryness score (Visual Analog Scale [VAS]) at Day 29.

Table 2 Summary of Primary Efficacy Endpoints

	CYS-002	**CYS-003**	**CYS-004**
Sign Endpoint	Change from baseline in total corneal fluorescein staining score (NEI scale) at Day 113	Change from baseline in total corneal fluorescein staining score (NEI scale) at Day 29	Change from baseline in total corneal fluorescein staining score (NEI scale) at Day 29
Symptom Endpoint	Change from baseline in dryness severity (VAS scale) at Day 113	Change from baseline in total OSDI score at Day 29	Change from baseline in dryness score (VAS scale) at Day 29
Post-hoc Analysis Endpoint	-	Proportion of responders in unanesthetized Schirmer's tear test (≥ 10 mm increase) at Day 85	Proportion of responders in unanesthetized Schirmer's tear test (≥ 10 mm increase) at Day 29

The primary symptom endpoints for the two studies CYS-003 and CYS-004 were different, because the efficacy result of the primary symptom endpoint in CYS-003 was not statistically significant, therefore, the results from CYS-003 guided the selection of the primary symptom endpoint for CYS-004. Accordingly, the two studies used different inclusion criteria: Study CYS-003 used an inclusion criterion based on the OSDI score and no inclusion criterion based on dryness score, while Study CYS-004 used an inclusion criterion based on dryness score and no inclusion criterion based on OSDI. In Study CYS-003, subjects were eligible if their OSDI score was ≥ 20 and if one eye met the following main inclusion criteria at screening and time of randomization: tCFS ≥ 10 (NEI scale), total lissamine green conjunctival score of ≥ 2 (Oxford

scale), and unanesthetized Schirmer's tear test score between 1 and 10 mm (inclusive). In Study CYS-004, subjects were eligible if they reported a dryness score ≥ 50 and if one eye met the following main inclusion criteria at screening and time of randomization: tCFS ≥ 10 (NEI scale), total lissamine green conjunctival score of ≥ 2 (Oxford scale), and Schirmer's tear test score between 1 and 10 mm (inclusive).

The FDA Draft Guidance for Industry on Dry Eye: Developing Drugs for Treatment (December 2020) recommends the sponsor to demonstrate efficacy and safety in at least two adequate and well-controlled, multicenter independent studies and to demonstrate one of the following:

- A statistically significant difference between the investigational treatment and vehicle for at least one objective prespecified sign of dry eye (mean group score of test versus vehicle) and at least one subjective prespecified symptom of dry eye (mean group score), or

- A statistically significant difference between the percentage of patients achieving a complete resolution of corneal staining, or

- A statistically significant difference between the percentage of patients achieving a 10-millimeter increase or more in Schirmer's tear test scores.

The Applicant's pre-specified primary endpoints were consistent with the first option, which was to demonstrate a significant difference between the test product and the vehicle in a sign endpoint and a symptom endpoint. However, the Applicant also conducted post-hoc analyses based on the proportion of patients achieving ≥ 10 mm increase in unanesthetized Schirmer's tear test (the third option in the draft guidance) in the two pivotal studies CYS-003 and CYS-004. It was specified as one of the secondary efficacy endpoints in the two studies.

3.2.2 Statistical Methodologies

For the three studies, the following analysis populations were defined:

- **Full Analysis Set (FAS)**: The FAS included all randomized subjects who received at least one dose of the study drug. The primary analysis was performed on the FAS and subjects in the FAS were analyzed as randomized.
- **Per Protocol Set (PPS)**: The PPS included subjects in the FAS who did not have major protocol deviations and who completed the trial. Subjects in the PPS were analyzed as treated.
- **Safety Set (SAF)**: The SAF included all randomized subjects who received at least one dose of the study drug. Subjects in the SAF were analyzed as treated.

In all three studies, the primary efficacy endpoints were analyzed using the analysis of covariance (ANCOVA) model. In CYS-004, the ANCOVA model included the terms for baseline value, site, and treatment group. In CYS-003, the ANCOVA model with the terms for baseline value, site, treatment group, and the interaction term of treatment by baseline value was considered. And the interaction term was maintained in the model only if the p-value for the interaction term was less

than 0.10. In the two pivotal studies CYS-003 and CYS-004, the two primary endpoints were tested using hierarchical fixed sequence testing (sign endpoint first and symptom endpoint second) to maintain the type I error rate. In the exploratory study CYS-002, the ANCOVA model included the terms for baseline value, treatment (including all 4 treatment groups), and the interaction of treatment by baseline value.

For the missing data, the SAPs of CYS-002 and CYS-003 stated that the primary analysis would use the FAS with available data per subject. The SAP of CYS-004 stated that the primary analysis would be completed with available data per subject with the FAS assuming the rate of missing data for an endpoint is less than 5%. If the rate of missing data for an endpoint is $\geq$ 5%, the primary analysis would be based on multiple imputation (if the missing data is balanced between treatment groups) or trimmed means (Permutt et al., 2017) (if the missing data is imbalanced between treatment groups). The amount of missing data in both primary endpoints of CYS-004 was less than 5%, therefore the Applicant conducted the primary analysis using FAS with available data. The summary of missing data in the two pivotal studies is provided in Table 16 and Table 17 of Appendix 2. As the percentage of subjects with missing data was about 3% in both pivotal studies, the impact of missing data was expected to be minimal. As sensitivity analyses, the analysis on the PPS and the analysis on the FAS using Last Observation Carried Forward (LOCF) approach were conducted.

For the post-hoc analysis of the proportion of responders in Schirmer's tear test, the Applicant reported the p-values for testing the proportion difference based on logistic regression with treatment as a sole covariate in Studies CYS-003 and CYS-004. According to the SAP of CYS-003, the percentage of Schirmer's tear test responders was a secondary efficacy endpoint and the responder percentage in each treatment group was to be compared using Fisher's Exact Test by visit. The CYS-004 SAP stated that as a dichotomous secondary endpoint, the proportion of Schirmer's tear test responders was to be analyzed using a logistic regression model with terms for baseline value, site, and treatment group. However, the Applicant stated in the CSR of CYS-004 that the logistic regression with baseline, site and treatment group led to a lack of model convergence, therefore the Applicant used the logistic regression model with only treatment factor to produce the statistical results and the same model was used for analyzing CYS-003. In this review, the proportion difference between the two treatment groups was tested using Normal approximation, and the results for Fisher's Exact Test and logistic regression with a factor of treatment group were also presented.

In all three studies, for efficacy endpoints that were measured separately in each eye, the unit of analysis was the "study eye." Eyes were eligible for analysis if they met all inclusion criteria and none of the exclusion criteria. In the case that both eyes were eligible for analysis, the study eye was the eye with the higher mean tCFS score at Visit 1. If the mean tCFS score was the same for both eyes, then the right eye was designated as the study eye.

Baseline measures were defined as the last non-missing measure prior to the initiation of the randomized study treatment. In most cases, this was the data from Visit 1.

3.2.3 Patient Disposition, Demographic and Baseline Characteristics

Studies CYS-002, CYS-003, and CYS-004 each randomized 207, 328, and 834 subjects, respectively. Table 3 summarizes the number of subjects in each analysis population by study and by treatment.

Table 3 Summary of Analysis Populations (All Randomized)

	CYS-002				CYS-003		CYS-004	
	CyclASol 0.05% (N=51)	**CyclASol 0.1% (N=51)**	**Restasis (N=53)**	**Vehicle (N=52)**	**CyclASol 0.1% (N=162)**	**Vehicle (N=166)**	**CyclASol 0.1% (N=423)**	**Vehicle (N=411)**
FAS	51 (100.0%)	51 (100.0%)	53 (100.0%)	52 (100.0%)	162 (100.0%)	166 (100.0%)	423 (100.0%)	411 (100.0%)
PPS	47 (92.2%)	49 (96.1%)	49 (92.5%)	47 (90.4%)	152 (93.8%)	161 (97.0%)	405 (95.7%)	391 (95.1%)
SAF	51 (100.0%)	51 (100.0%)	53 (100.0%)	52 (100.0%)	162 (100.0%)	166 (100.0%)	423 (100.0%)	411 (100.0%)

Source: Table 5 of CYS-002 CSR, Table 5 of CYS-003 CSR, and Table 4 of CYS-004 CSR

Table 4 shows the subject disposition. For all three studies, a small percentage of randomized subjects (less than 5%) discontinued early except for the vehicle arm of CYS-002 (7.7% of subjects discontinued). In CYS-003, one subject (Subject 41-033) in CyclASol arm was classified as discontinued due to subject choice, but according to the Applicant's Adverse Event Listing (16.2.7), the subject had adverse events that were suspected to have relationship to the study drug, leading to treatment discontinuation. CYS-003 had slightly higher percentage of discontinued subjects for CyclASol than for the vehicle (4.3% vs. 1.8%), and CYS-004 had comparable percentages of discontinued subjects for both treatment groups (1.9% vs. 2.2%).

Table 4 Subject Disposition (FAS)

	CYS-002				CYS-003		CYS-004	
	CyclASol 0.05% (N=51)	**CyclASol 0.1% (N=51)**	**Restasis (N=53)**	**Vehicle (N=52)**	**CyclASol 0.1% (N=162)**	**Vehicle (N=166)**	**CyclASol 0.1% (N=423)**	**Vehicle (N=411)**
Completed Study	50 (98.0%)	50 (98.0%)	52 (98.0%)	48 (92.3%)	155 (95.7%)	163 (98.2%)	415 (98.1%)	402 (97.8%)
Discontinued	1 (2.0%)	1 (2.0%)	1 (1.9%)	4 (7.7%)	7 (4.3%)	3 (1.8%)	8 (1.9%)	9 (2.2%)
Discontinuations Related to Covid-19	-	-	-	-	-	-	0	0
Reasons for Discontinuation								
Adverse Events	1 (2.0%)	0	1 (1.9%)	2 (3.8%)	2 (1.2%)	0	2 (0.5%)	0
Administrative Reasons	0	0	0	0	2 (1.2%)	2 (1.2%)	0	0
Lost to Follow Up	0	0	0	0	0	0	0	3 (0.7%)
Subject Choice	0	0	0	2 (3.8%)	3 (1.9%)	0	5 (1.2%)	5 (1.2%)
Other	0	1 (2.0%)	0	0	0	1 (0.6%)	1 (0.2%)	1 (0.2%)

Source: Table 5 of CYS-002 CSR, Table 5 of CYS-003 CSR, and Table 4 of CYS-004 CSR

Table 5 shows the summary of demographic and baseline characteristics. Demographic and baseline disease characteristics were generally comparable between treatment groups in the three studies. Most subjects were female (over 70%), not Hispanic (over 80%), and white (over 70%). The mean age in the three studies was approximately 60 years old (range from 18 to 94).

Table 5 Demographic and Baseline Characteristics (FAS)

	CYS-002				**CYS-003**		**CYS-004**	
	CyclASol 0.05% (N=51)	**CyclASol 0.1% (N=51)**	**Restasis (N=53)**	**Vehicle (N=52)**	**CyclASol 0.1% (N=162)**	**Vehicle (N=166)**	**CyclASol 0.1% (N=423)**	**Vehicle (N=411)**
Age (years)								
Mean (SD)	64.3 (10.72)	61.1 (12.29)	62.8 (11.90)	61.3 (10.45)	61.5 (13.60)	61.3 (12.66)	57.6 (15.36)	56.6 (16.30)
Median	64.0	61.0	62.0	62.0	62.0	63.0	60.0	59.0
Min, Max	33, 85	30, 86	27, 94	26, 82	18, 93	19, 89	19, 86	18, 93
Age category, n (%)								
18 to < 65 years	26 (51.0%)	32 (62.7%)	35 (66.0%)	31 (59.6%)	99 (61.1%)	91 (54.8%)	266 (62.9%)	261 (63.5%)
≥ 65 years	25 (49.0%)	19 (37.3%)	18 (34.0%)	21 (40.4%)	63 (38.9%)	75 (45.2%)	157 (37.1%)	150 (36.5%)
Gender, n (%)								
Female	38 (74.5%)	36 (70.6%)	40 (75.5%)	39 (75.0%)	116 (71.6%)	119 (71.7%)	306 (72.3%)	303 (73.7%)
Male	13 (25.5%)	15 (29.4%)	13 (24.5%)	13 (25.0%)	46 (28.4%)	47 (28.3%)	117 (27.7%)	108 (26.3%)
Ethnicity, n (%)								
Hispanic or Latino	1 (2.0%)	3 (5.9%)	2 (3.8%)	2 (3.8%)	15 (9.3%)	11 (6.6%)	62 (14.7%)	60 (14.6%)
Not Hispanic or Latino	50 (98.0%)	48 (94.1%)	51 (96.2%)	50 (96.2%)	147 (90.7%)	155 (93.4%)	359 (84.9%)	348 (84.7%)
Unknown or not reported	0	0	0	0	0	0	2 (0.5%)	3 (0.7%)
Race, n (%)								
American Indian or Alaska Native	0	0	0	0	1 (0.6%)	1 (0.6%)	2 (0.5%)	0
Asian	0	2 (3.9%)	2 (3.8%)	0	14 (8.6%)	19 (11.4%)	40 (9.5%)	39 (9.5%)
Black or African American	0	5 (9.8%)	5 (9.4%)	5 (9.6%)	16 (9.9%)	14 (8.4%)	53 (12.5%)	55 (13.4%)
Native Hawaiian or Other Pacific Islander	1 (2.0%)	0	0	0	1 (0.6%)	1 (0.6%)	1 (0.2%)	0
White	50 (98.0%)	44 (86.3%)	46 (86.8%)	47 (90.4%)	130 (80.2%)	127 (76.5%)	323 (76.4%)	312 (75.9%)
Other	0	0	0	0	0	1 (0.6%)	1 (0.2%)	1 (0.2%)
Multiple race	0	0	0	0	0	3 (1.8%)	2 (0.5%)	3 (0.7%)
Unknown	0	0	0	0	0	0	0	1 (0.2%)
Not reported	0	0	0	0	0	0	1 (0.2%)	0
Disease Duration (years)								
Mean (SD)	12.5 (10.48)	11.1 (9.85)	10.5 (7.40)	11.7 (9.20)	12.2 (10.60)	12.3 (10.69)	10.3 (9.55)	10.4 (10.32)

Median	9.1	8.7	10.0	10.0	10.0	10.1	6.4	6.3
Min, Max	2, 51	1, 46	1, 36	2, 42	1, 73	1, 48	1, 65	1, 55
tCFS (0-15)								
Mean (SD)	8.8 (2.14)	8.8 (1.89)	8.8 (1.89)	8.7 (2.15)	11.5 (1.26)	11.5 (1.25)	11.5 (1.41)	11.5 (1.36)
Median	9.0	9.0	9.0	9.0	11.0	11.0	11.0	11.0
Min, Max	6, 14	6, 13	6, 14	6, 14	10, 15	10, 15	10, 15	8, 15
Dryness Score VAS (0-100)								
Mean (SD)	72.8 (19.55)	71.2 (16.17)	68.5 (18.80)	68.5 (21.38)	68.5 (21.65)	69.9 (20.46)	70.4 (12.53)	70.0 (12.59)
Median	80.0	71.0	70.0	70.5	74.0	74.0	70.0	70.0
Min, Max	20, 100	35, 100	30, 100	10, 100	3, 100	0, 100	50, 100	50, 100
Total OSDI (0-100)								
Mean (SD)	38.02 (18.653)	40.37 (19.317)	35.38 (16.641)	37.91 (16.477)	46.94 (16.732)	47.13 (16.408)	47.06 (21.066)	47.15 (19.290)
Median	34.09	37.50	35.00	36.93	44.10	47.61	47.50	47.50
Min, Max	0, 79.2	4.5, 85.0	2.1, 77.3	7.5, 77.1	20.0, 93.8	20.5, 90.0	0.0, 100.0	0.0, 93.2
Schirmer's tear test (mm)								
Mean (SD)	5.1 (2.13)	5.2 (2.16)	4.4 (1.90)	4.6 (2.33)	5.3 (2.82)	5.1 (2.64)	5.1 (2.96)	4.8 (2.78)
Median	5.0	5.0	4.0	4.5	5.0	5.0	5.0	4.0
Min, Max	2, 8	2, 8	2, 8	2, 8	1, 10	1, 10	1, 10	1, 10

Source: Tables 8 & 9 of CYS-002 CSR, Tables 7 & 8 of CYS-003 CSR, Table 5 & 6 of CYS-004 CSR, and Reviewer's Analysis

3.2.4 Results and Conclusions

3.2.4.1 Results of Phase 2 Study CYS-002

Table 6 shows the summary of the primary efficacy results of Study CYS-002. As it was an exploratory Phase 2 study, there were no formal hypothesis tests and no adjustments for multiple comparisons.

Regarding the primary sign endpoint, both CyclASol 0.05% and CyclASol 0.1% groups had numerically better improvement in tCFS at Day 113 than the vehicle group. Regarding the primary symptom endpoint, neither CyclASol 0.05% nor CyclASol 0.1% had better improvement in dryness score at Day 113 compared to the vehicle. On the other hand, CyclASol 0.1% group had a numerically better improvement than the vehicle group in total OSDI score, which was one of the secondary endpoints. This result led the Applicant to change the primary symptom endpoint of the subsequent study, CYS-003, to change from baseline in total OSDI score.

Table 6 Summary of Primary Efficacy Results of Study CYS-002 (FAS)

		CyclASol 0.05% (N=51)	CyclASol 0.1% (N=51)	Restasis (N=53)	Vehicle (N=52)	CyclASol 0.05% – Vehicle	CyclASol 0.1% – Vehicle	Restasis – Vehicle
		Mean Change from Baseline				LS Mean Difference (95% CI)		
Primary Sign Endpoint	**tCFS at Day 113**	-2.38	-2.10	-1.56	-1.69	-0.68 (-1.57, 0.21)	-0.42 (-1.32, 0.47)	0.14 (-0.74, 1.03)
Primary Symptom Endpoint	**Dryness score at Day 113**	-11.00	-9.30	-13.85	-19.00	2.45 (-7.18, 12.08)	4.27 (-5.36, 13.90)	-5.54 (-15.08, 4.00)
Secondary Efficacy Endpoint	**Total OSDI score at Day 113**	-1.99	-6.84	-8.09	-3.69	1.66 (-3.76, 7.07)	-2.74 (-8.16, 2.69)	-5.22 (-10.60, 0.15)

Source: Table 24 of Summary of Clinical Efficacy, Table 47 and Table 14.2.9.1 of CYS-002 CSR

3.2.4.2 Results of Primary Sign Endpoint

Table 7 presents the efficacy results for the primary sign endpoint, the change from baseline in tCFS score (NEI scale) at Day 29, in the two pivotal studies CYS-003 and CYS-004.

Both studies CYS-003 and CYS-004 demonstrated superiority of CyclASol 0.1% to the vehicle regarding the primary sign endpoint. In CYS-003, the least squares (LS) mean change from baseline in tCFS at Day 29 was -2.11 for the CyclASol group and -1.68 for the vehicle group. The estimated treatment difference (CyclASol – Vehicle) was -0.42 with the 95% confidence interval of (-1.28, -0.40) and the p-value of 0.0002. In CYS-004, the LS mean change from baseline in tCFS at Day 29 was -3.96 for CyclASol group and -3.55 for vehicle group. The estimated treatment difference was -0.41 with the 95% confidence interval of (-0.77, -0.04) and the p-value of 0.0278.

Table 7 Results for the Primary Sign Endpoint, Using FAS with Available Data

		CyclASol 0.1% (N=162)	Vehicle (N=166)
CYS-003	**Change from baseline in tCFS at Day 29**		
	n	157	165
	Mean (SD)	-2.93 (2.59)	-2.18 (2.73)
	Median (Min, Max)	-3 (-11, 2)	-2 (-10, 3)
	Adjusted Mean Change from Baseline in tCFS Day 29 (ANCOVA)*		
	LS Mean (SE)	-3.49 (0.171)	-2.65 (0.166)
	LS Mean Difference (SE)	-0.840 (0.225)	
	95% CI	(-1.2830, -0.3976)	
	p-value	0.0002	
		CyclASol 0.1% (N=423)	**Vehicle (N=411)**
CYS-004	**Change from baseline in tCFS at Day 29**		
	n	409	395
	Mean (SD)	-4.33 (3.15)	-3.91 (3.44)
	Median (Min, Max)	-4 (-13, 5)	-3 (-13, 4)
	Adjusted Mean Change from Baseline in tCFS at Day 29 (ANCOVA)*		
	LS Mean (SE)	-3.96 (0.149)	-3.55 (0.146)
	LS Mean Difference (SE)	-0.409 (0.186)	
	95% CI	(-0.7742, -0.0447)	
	p-value	0.0278	

* The result is based on ANCOVA model with baseline value as covariate and site and treatment as factors.

The primary sign endpoint analysis results in the two pivotal studies were robust against different strategies to handle missing data (Table 18 in Appendix 3).

The results for the primary sign endpoint were consistent for the other visits in the two studies. At the visits other than Day 29 (Visit 3) in both studies, the CyclASol group showed consistently better improvement in tCFS score than the vehicle group (Table 21 and Figure 4 in Appendix 4). Figure 4 in Appendix 4 shows the estimated treatment means of the change from baseline in tCFS with the 95% confidence intervals over visits in the two studies. Both the CyclASol group and the vehicle group showed continuous reduction in tCFS score over visits with more reduction for the CyclASol group.

3.2.4.3 Results of Primary Symptom Endpoint of CYS-003

Table 8 presents the efficacy results for the primary symptom endpoint of Study CYS-003, the change from baseline in total OSDI score at Day 29, in the two pivotal studies CYS-003 and CYS-004.

Neither of the two studies demonstrated superiority of CyclASol to the vehicle regarding the change from baseline in total OSDI score.

In Study CYS-003, the CyclASol group had a numerically better improvement in total OSDI score than the vehicle group, although the difference was not statistically significant. The LS mean change from baseline in OSDI score was -8.76 for the CyclASol group and -6.81 for the vehicle

group, with the estimated treatment difference of -1.95, the 95% confidence interval of (-5.38, 1.48) and the p-value of 0.2634.

In Study CYS-004, the CyclASol group did not show a better improvement in total OSDI score than the vehicle group. The LS mean change from baseline in total OSDI was -6.13 for CyclASol and -7.13 for the vehicle, indicating a greater reduction in total OSDI for the vehicle.

Table 8 Results for the Symptom Endpoint of Change from Baseline in Total OSDI at Day 29 for CYS-003 and CYS-004, Using FAS with Available Data

		CyclASol 0.1% (N=162)	**Vehicle (N=166)**
CYS-003 (Primary Efficacy Endpoint)	**Change from baseline in Total OSDI at Day 29**		
	n	157	165
	Mean (SD)	-7.08 (18.65)	-5.37 (15.29)
	Median (Min, Max)	-6.06 (-66.67, 65.91)	-6.25 (-52.08, 31.25)
	Adjusted Mean Change from Baseline in Total OSDI at Day 29 (ANCOVA) *		
	LS Mean (SE)	-8.76 (1.33)	-6.81 (1.29)
	LS Mean Difference (SE)	-1.95 (1.74)	
	95% CI	(-5.38, 1.48)	
	p-value	0.2634	
		CyclASol 0.1% (N=423)	**Vehicle (N=411)**
CYS-004 (Exploratory Efficacy Endpoint)	**Change from baseline in Total OSDI at Day 29**		
	n	409	395
	Mean (SD)	-6.02 (16.89)	-7.21 (17.60)
	Median (Min, Max)	-4.64 (-64.39, 41.29)	-6.25 (-60.42, 68.75)
	Adjusted Mean Change from Baseline in Total OSDI at Day 29 (ANCOVA)*		
	LS Mean (SE)	-6.13 (0.89)	-7.36 (0.91)
	LS Mean Difference (SE)	1.23 (1.14)	
	95% CI	(-1.00, 3.46)	
	p-value	0.2791	

* The result is based on ANCOVA model with baseline value as covariate and site and treatment as factors.

The results for the primary symptom endpoint regarding the total OSDI score were consistent for the other visits in the two studies (Table 22 and Figure 5 in Appendix 4). None of the visits in both studies showed superiority of CyclASol to the vehicle regarding the change from baseline in total OSDI score.

3.2.4.4 Results of Primary Symptom Endpoint of CYS-004

Table 9 presents the efficacy results for the primary symptom endpoint of Study CYS-004, the change from baseline in dryness score (VAS scale) at Day 29, in the two pivotal studies CYS-003 and CYS-004.

Although this endpoint was not pre-specified as the primary symptom endpoint for Study CYS-003, Study CYS-003 demonstrated superiority of CyclASol to the vehicle regarding the change from baseline in dryness score at Day 29. The LS mean change from baseline in dryness score was

-10.71 for the CyclASol group and -5.92 for the vehicle group. The estimated treatment difference was -4.78 with the 95% confidence interval of (-9.13, -0.44) and the p-value of 0.0311.

However, Study CYS-004 failed to demonstrate superiority of CyclASol to the vehicle regarding its primary symptom endpoint. The LS mean change from baseline in dryness score was -12.19 for the CyclASol group and -13.62 for the vehicle group, indicating a greater reduction in dryness score for the vehicle group.

Table 9 Results for the Symptom Endpoint of Change from Baseline in Dryness Score at Day 29 for CYS-003 and CYS-004, Using FAS with Available Data

		CyclASol 0.1% (N=162)	**Vehicle (N=166)**
CYS-003 (Secondary Efficacy Endpoint)	**Change from baseline in Dryness Score at Day 29**		
	n	157	165
	Mean (SD)	-9.32 (22.18)	-5.35 (20.60)
	Median (Min, Max)	-10 (-70, 60)	-1 (-75, 90)
	Adjusted Mean Change from Baseline in Dryness Score at Day 29 (ANCOVA)*		
	LS Mean (SE)	-10.71 (1.68)	-5.92 (1.63)
	LS Mean Difference (SE)	-4.78 (2.21)	
	95% CI	(-9.1285, -0.4383)	
	p-value	0.0311	
		CyclASol 0.1% (N=423)	**Vehicle (N=411)**
CYS-004 (Primary Efficacy Endpoint)	**Change from baseline in Dryness Score at Day 29**		
	n	409	395
	Mean (SD)	-12.64 (23.72)	-13.70 (23.41)
	Median (Min, Max)	-10 (-87, 46)	-10 (-89, 41)
	Adjusted Mean Change from Baseline in Dryness Score at Day 29 (ANCOVA)*		
	LS Mean (SE)	-12.19 (1.29)	-13.62 (1.31)
	LS Mean Difference (SE)	1.43 (1.64)	
	95% CI	(-1.7891, 4.6409)	
	p-value	0.3842	

* The result is based on ANCOVA model with baseline value as covariate and site and treatment as factors.

The primary analysis results for change from baseline in dryness score were robust against different strategies to handle missing data (Table 19 in Appendix 3).

Table 23 and Figure 6 in Appendix 4 show the analysis results for the change from baseline in dryness score at the visits other than Day 29 (Visit 3) in the two studies. None of those visits in both studies showed superiority of CyclASol to the vehicle regarding the change from baseline in dryness score.

3.2.4.5 Results of Post-hoc Analysis Endpoint

Table 10 presents the efficacy results for the post-hoc analysis endpoint, the proportion of responders in unanesthetized Schirmer's tear test (≥ 10 mm increase) in the three studies, CYS-002, CYS-003 and CYS-004. As this endpoint was analyzed as a post-hoc analysis, it was not pre-specified which time point the efficacy should be evaluated at, and therefore all available data (all

visits where Schirmer's tear test was assessed) were analyzed. The Applicant chose to use Day 85 for Study CYS-003 and Day 29 for Study CYS-004 to draw an efficacy conclusion.

In Study CYS-003, for the initial three post-baseline visits (Day 15, Day 29, and Day 57), the CyclASol group showed a numerically higher proportion of responders than the vehicle group (8.1% vs. 3.7% for Day 15; 4.5% vs. 4.2% for Day 29; 5.8% vs. 4.3% for Day 57) although the proportion difference was not statistically significant at those three visits. Day 85 was the only visit where the CyclASol group had a significantly higher proportion of responders than the vehicle group (12.3% vs. 5.5%, p-value = 0.0341).

In Study CYS-004, Day 29 was the only post-baseline visit where Schirmer's tear test was assessed. At Day 29, the CyclASol group showed a higher proportion of responders than the vehicle group with marginal significance (10.8% vs. 6.8%, p-value = 0.0500).

The time trend of the proportion of Schirmer's tear test responders over visits in Studies CYS-003 and CYS-002 was examined (Table 10 and Figure 7 in Appendix 4). The reason that the Applicant chose Day 85 for evaluating this endpoint in CYS-003 was that the increase in tear production, which is a known effect of cyclosporine, typically takes time to develop. However, in Studies CYS-003 and CYS-002, the proportion of Schirmer's tear test responders had a fluctuating trend over visits, which does not provide appropriate information on at which time point the increase in tear production have been developed and at which time point the endpoint should have been evaluated. We acknowledge that the sizes of the two studies are too small to evaluate the trend over visits. Nonetheless, it should be noted that the study results do not provide reliable evidence of the gradual effect of CyclASol 0.1% on the increase in tear production.

Table 10 Results for the Post-hoc Analysis Endpoint (Proportion of Schirmer's Tear Test Responders), Using FAS with Available Data

		CYS-002		CYS-003		CYS-004	
		CyclASol 0.1% (N=51)	Vehicle (N=52)	CyclASol 0.1% (N=162)	Vehicle (N=166)	CyclASol 0.1% (N=423)	Vehicle (N=411)
Day 15	n	51	51	160	164		
	Responders, n (%)	1 (1.96%)	0 (0.00%)	13 (8.12%)	6 (3.66%)		
	Difference (95% CI) [1]	1.96% (-1.84%, 5.77%)		4.47% (-0.65%, 9.58%)			
	p-value[1]	0.3149		0.0871			
	Odds Ratio (95% CI)[2]	-		2.33 (0.90, 6.78)			
	p-value[2]	-		0.0952			
	p-value[3]	1		0.1012			
Day 29	n	51	50	157	165	409	395
	Responders, n (%)	4 (7.84%)	0 (0.00%)	7 (4.46 %)	7 (4.24 %)	44 (10.76%)	27 (6.84%)
	Difference (95% CI) [1]	7.84% (0.46%, 15.22%)		0.22% (-4.24%, 4.67%)		3.92 % (0.02%, 7.82%)	
	p-value[1]	0.0433		0.9243		0.0500	
	Odds Ratio (95% CI)[2]	-		1.05 (0.35, 3.15)		1.64 (1.00, 2.74)	
	p-value[2]	-		0.9243		0.0519	
	p-value[3]	0.1176		1		0.0617	
Day 57	n			156	164		
	Responders, n (%)			9 (5.77%)	7 (4.27%)		
	Difference (95% CI) [1]			1.50% (-3.29%, 6.29%)			
	p-value[1]			0.5380			
	Odds Ratio (95% CI)[2]			1.37 (0.50, 3.93)			
	p-value[2]			0.5395			

	p-value[3]			0.6131		
Day 85	n	49	50	155	163	
	Responders, n (%)	2 (4.08%)	5 (10.00%)	19 (12.26%)	9 (5.52%)	
	Difference (95% CI) [1]	-5.92% (-15.91%, 4.07%)		6.74% (0.50%, 12.98%)		
	p-value[1]	0.2507		0.0341		
	Odds Ratio (95% CI)[2]	0.38 (0.05, 1.88)		2.39 (1.07, 5.71)		
	p-value[2]	0.2656		0.0386		
	p-value[3]	0.4360		0.0465		
Day 113	n	50	48			
	Responders, n (%)	3 (6.00%)	3 (6.25%)			
	Difference (95% CI) [1]	-0.25% (-9.75%, 9.25%)				
	p-value[1]	0.9588				
	Odds Ratio (95% CI)[2]	0.96 (0.17, 5.41)				
	p-value[2]	0.9588				
	p-value[3]	1				

[1] The 95% CI and p-value are based on Normal approximation.
[2] The odds ratio, 95% CI and p-value are calculated using logistic regression model with a factor for treatment group.
[3] The p-value is based on Fisher's exact test.
The greyed cells indicate the visits where Schirmer's tear test measurements were not available.

3.3 Evaluation of Safety

In this section, a high-level summary of safety evaluation for the individual studies CYS-002, CYS-003, CYS-004 and CYS-005 is provided. A comprehensive safety evaluation is covered in the FDA clinical review. Study CYS-005, which was not reviewed in the evaluation of efficacy, was an extension of Study CYS-004 aiming to investigate the long-term safety and efficacy of CyclASol 0.1% in subjects with dry eye disease. Subjects, who completed CYS-004 and were eligible and willing to continue in CYS-005, received CyclASol 0.1% for 12 months.

Table 11 shows the summary of exposure to study drug for the four studies CYS-002, CYS-003, CYS-004, and CYS-005. In each study, the duration of exposure to study drug was comparable between the treatment groups.

Table 11 Exposure to Study Drug (in Days)

	CyclASol 0.05%				**CyclASol 0.1%**				**Vehicle**			
Studies	**N**	**Mean (SD)**	**Median**	**Range**	**N**	**Mean (SD)**	**Median**	**Range**	**N**	**Mean (SD)**	**Median**	**Range**
CYS-002	51	110.7 (15.57)	113.0	2, 116	51	111.2 (11.93)	113.0	28, 115	52	109.0 (19.22)	113.0	1, 115
CYS-003	-	-	-	-	162	82.9 (12.05)	85.0	5, 98	166	84.2 (9.00)	85.0	1, 117
CYS-004	-	-	-	-	423	29.0 (3.04)	29.0	3, 54	411	28.9 (3.83)	29.0	1, 69
					CyclASol 0.1% (CYS-004) to CyclASol 0.1%				**Vehicle (CYS-004) to CyclASol 0.1%**			
CYS-005	-	-	-	-	98	329.6 (93.29)	365.0	22, 381	102	339.5 (73.48)	365.0	29, 394

Source: Table 14.3.8 of CYS-002 CSR, Table 14.3.7 of CYS-003 CSR, Table 15 of CYS-004 CSR, and Table 12-1 of CYS-005 CSR

Table 12 presents the summary of adverse events in the pooled safety population of the four studies CYS-002, CYS-003, CYS-004, and CYS-005. Overall, the CyclASol 0.1% group had slightly higher incidences of adverse events (AEs), treatment-emergent adverse events (TEAEs),

ocular/non-ocular TEAEs than the vehicle group, which may reflect the longer durations of the studies that contribute to the CyclASol 0.1% group. There were no adverse events resulting in death.

Table 12 Summary of Adverse Events (Pooled SAF: CYS-002, CYS-003, CYS-004, and CYS-005)

	CyclASol 0.05% (N=51)	CyclASol 0.1% (N=738)	Vehicle (N=629)
AEs	19 (37.3%)	225 (30.5%)	137 (21.8%)
TEAEs	18 (35.3%)	217 (29.4%)	131 (20.8%)
Ocular TEAEs	7 (13.7%)	133 (18.0%)	84 (13.4%)
Non-Ocular TEAEs	13 (25.5%)	115 (15.6%)	58 (9.2%)
TE-SAEs	2 (3.9%)	6 (0.8%)	6 (1.0%)
TEAEs with Relationship to Study Drug Suspected	2 (3.9%)	77 (10.4%)	55 (8.7%)
AEs Leading to Treatment Discontinuation	1 (2.0%)	8 (1.1%)	4 (0.6%)
AEs Resulting in Death	0	0	0

- AE = adverse event, TEAE = treatment-emergent adverse event, TE-SAE = treatment emergent serious adverse event.
- Note: Subjects who participated in CYS-005 and who received vehicle in CYS-004 are counted in both the CyclASol 0.1% and Vehicle treatment arms.

Source: Table 4 of Summary of Clinical Safety

Table 13 presents the summary of the most common ocular TEAEs with > 2 subjects per study. The overall incidences of ocular TEAEs were comparable between the three studies CYS-002, CYS-003, and CYS-004 (about 13%), while the incidence of ocular TEAEs in CYS-005 was higher (27.5%) compared to the other three studies. At the system organ class (SOC) level, the most common ocular TEAEs in Studies CYS-002, CYS-003, and CYS-005 were eye disorders. The most common ocular TEAEs in Study CYS-004 were general disorders and administration site conditions.

Table 13 Most Common Ocular TEAEs (> 2 subjects per study, SAF) in Individual Studies CYS-002, CYS-003, CYS-004, and CYS-005

	CYS-002			CYS-003		CYS-004		CYS-005
System Organ Class (SOC) Preferred Term (PT)	**CyclASol 0.05% (N=51)**	**CyclASol 0.1% (N=51)**	**Vehicle (N=52)**	**CyclASol 0.1% (N=162)**	**Vehicle (N=166)**	**CyclASol 0.1% (N=423)**	**Vehicle (N=411)**	**All Subjects (N=200)**
Number of subjects with ≥ 1 ocular TEAE	**7 (13.7%)**	**7 (13.7%)**	**8 (15.4%)**	**20 (12.3%)**	**14 (8.4%)**	**57 (13.5%)**	**62 (15.1%)**	**55 (27.5%)**
General disorders and administration site conditions	**1 (2.0%)**	**1 (2.0%)**	**2 (3.8%)**	**4 (2.5%)**	**3 (1.8%)**	**43 (10.2%)**	**36 (8.8%)**	**14 (7.0%)**
Instillation site pain	1 (2.0%)	1 (2.0%)	1 (1.9%)	4 (2.5%)	2 (1.2%)	42 (9.9%)	35 (8.5%)	13 (6.5%)
Eye disorders	**6 (11.8%)**	**6 (11.8%)**	**4 (7.7%)**	**16 (9.9%)**	**12 (7.2%)**	**18 (4.3%)**	**28 (6.8%)**	**36 (18.0%)**
Visual acuity reduced	2 (3.9%)	4 (7.8%)	1 (1.9%)	5 (3.1%)	3 (1.8%)	7 (1.7%)	13 (3.2%)	6 (3.0%)
Vision blurred	0	1 (2.0%)	0	2 (1.2%)	4 (2.4%)	2 (0.5%)	2 (0.5%)	2 (1.0%)
Vitreous detachment	0	0	0	0	0	1 (0.2%)	0	9 (4.5%)
Eye irritation	0	0	2 (3.8%)	2 (1.2%)	0	0	3 (0.7%)	1 (0.5%)
Eye pruritus	0	1 (2.0%)	0	1 (0.6%)	1 (0.6%)	1 (0.2%)	2 (0.5%)	0
Dry eye	0	0	0	0	0	1 (0.2%)	3 (0.7%)	1 (0.5%)
Eyelid margin crusting	0	0	0	2 (1.2%)	2 (1.2%)	0	0	0

Vitreous floaters	0	0	0	2 (1.2%)	1 (0.6%)	0	1 (0.2%)	0
Posterior capsule opacification	0	0	0	0	0	0	0	3 (1.5%)
Infections and infestations	0	**1 (2.0%)**	**1 (1.9%)**	**2 (1.2%)**	**1 (0.6%)**	0	**1 (0.2%)**	**1 (0.5%)**
Hordeolum	0	0	0	2 (1.2%)	1 (0.6%)	0	0	0

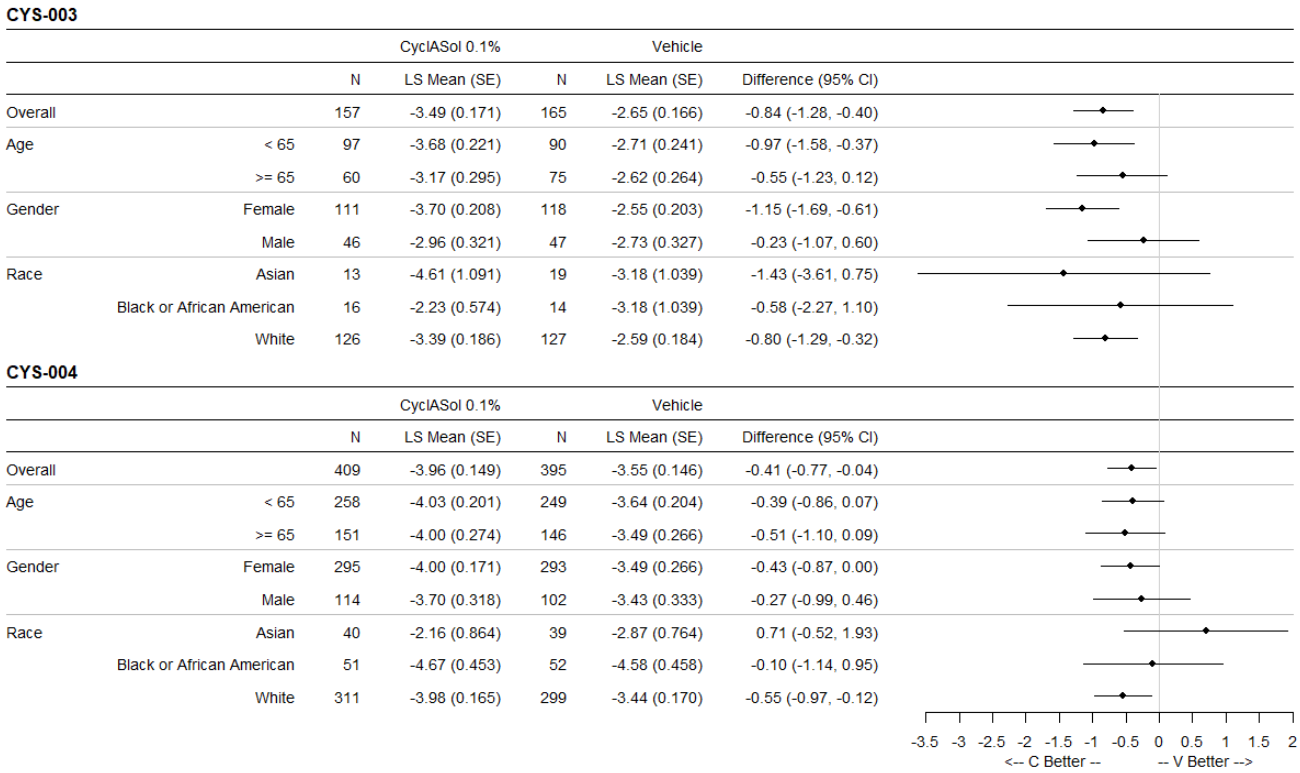
Source: Table 5 of Summary of Clinical Safety

4 FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

4.1 Gender, Race, Age, and Geographic Region

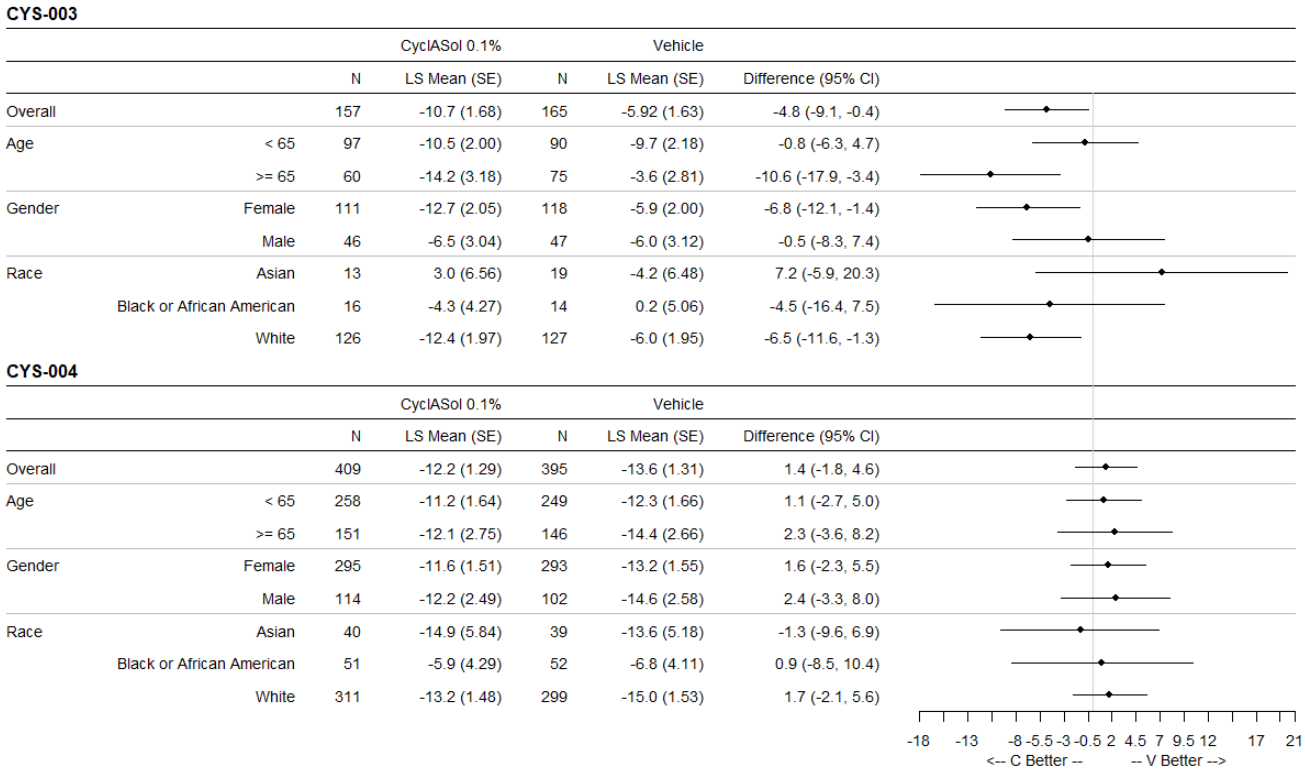
Subgroup analyses based on age group (< 65 vs. ≥ 65 years), gender, and race were performed by the Applicant. Figure 2 presents the subgroup analyses for the primary sign endpoint (change from baseline in tCFS score at Day 29) and Figure 3 presents the subgroup analyses for the primary symptom endpoint of Study CYS-004 (change from baseline in dryness score at Day 29). In both studies, the subgroup analysis results were similar to those seen for the overall population except for the Asian group (it had small sample sizes, 13 in CYS-003 and 40 in CYS-004).

Figure 2 Subgroup Analyses for the Primary Sign Endpoint (Change from Baseline in tCFS Score at Day 29) among Subgroups of Age, Gender, and Race, Using FAS with Available Data



The results are based on ANCOVA model with baseline value as covariate and site and treatment as factors.
Source: Tables 14.2.1.3.1.4a, 14.2.1.3.1.4b, 14.2.1.3.1.5a, 14.2.1.3.1.5b, 14.2.1.3.1.6a, 14.2.1.3.1.6b of IR response (Seq 0005)

Figure 3 Subgroup Analyses for the Primary Symptom Endpoint of CYS-004 (Change from Baseline in Dryness Score at Day 29) among Subgroups of Age, Gender, and Race, Using FAS with Available Data



The results are based on ANCOVA model with baseline value as covariate and site and treatment as factors.
Source: Tables 14.2.2.5.1.4a, 14.2.2.5.1.4b, 14.2.2.5.1.5a, 14.2.2.5.1.5b, 14.2.2.5.1.6a, 14.2.2.5.1.6b of IR response (Seq 0005)

4.2 Other Special/Subgroup Populations

No other subgroups were analyzed.

5 SUMMARY AND CONCLUSIONS

5.1 Statistical Issues

There were no major statistical issues in this NDA submission.

5.2 Collective Evidence

Both pivotal studies CYS-003 and CYS-004 demonstrated superiority of CyclASol 0.1% to the vehicle for the pre-specified primary sign endpoint of change from baseline in tCFS score at Day 29.

Both studies failed to demonstrate superiority of CyclASol 0.1% to the vehicle for their pre-specified co-primary symptom endpoint(s). Study CYS-003 showed a numeric trend in favor of the CyclASol group regarding its primary symptom endpoint, the change from baseline in total OSDI score at Day 29, but it failed to show a statistically significant difference. Study CYS-004 failed to show superiority of CyclASol to the vehicle regarding its primary symptom endpoint, the change from baseline in dryness score at Day 29. Study CYS-003 met this primary symptom endpoint of CYS-004 with the p-value of 0.0311, although it was not the pre-specified primary symptom endpoint. Furthermore, both studies did not demonstrate evidence for superiority of CyclASol 0.1% compared to vehicle with respect to the primary symptom endpoint(s) at all visits other than Day 29 (Visit 3).

The post-hoc analysis using the proportion of responders in Schirmer's tear test showed superiority of CyclASol 0.1% to the vehicle at Day 85 in Study CYS-003 and showed a marginally significant result in Study CYS-004.

5.3 Conclusions and Recommendations

In conclusion, based on the collective evidence of the two pivotal studies CYS-003 and CYS-004, the application provided statistically significant evidence that CyclASol 0.1% is superior to the vehicle in treating the signs of dry eye disease. However, both studies failed to provide adequate evidence for the superiority of CyclASol 0.1% to the vehicle in treating the symptoms of dry eye disease.

The Applicant's efficacy conclusion is based on the post-hoc analysis using the proportion of Schirmer's tear test responders. However, this analysis does not provide adequate evidence to support superiority of CyclASol 0.1% to the vehicle, because it was not a pre-specified analysis and the results are not robustly significant and lacks supportive evidence.

5.4 Labeling Recommendations (as applicable)

The Applicant's proposed label includes the summary of efficacy results based on:

- Percent of patients achieving ≥ 10 mm improvement from baseline in Schirmer's tear test score (Table 1 of the proposed label),

(b) (4)

We acknowledge that percent of Schirmer's tear test responders is a single primary endpoint recommended in the FDA Draft Guidance for Industry on Dry Eye: Developing Drugs for Treatment (December 2020). Yet, it could be misleading to present the efficacy results regarding

only the sign endpoints and omit the efficacy results for the symptom endpoints, while the study drug is indicated to treat the signs and symptoms of dry eye disease.

APPENDIX 1: Study Schedules

Table 14 Schedule of Visits and Measurements of Study CYS-003

Procedure	Visit 0 Day -14 ± 2	Visit 1 Day 1	Visit 2 Day 15 ± 1	Visit 3 Day 29 ± 2	Visit 4 Day 57 ± 2	Visit 5 Day 85 ± 2
Informed Consent / HIPAA	X					
Medical / Medication History and Demographics	X					X
Urine Pregnancy Test (as needed)	X					X
Medical/Medication History Update		X	X	X	X	X
Adverse Event Query	X	X	X	X	X	X
OSDI	X	X	X	X	X	X
Lead/Worst symptom selection and assessment		X	X	X	X	X
Reading impairment score	X	X	X	X	X	X
VAS	X	X	X	X	X	X
Reading Assessments		X		X		X
Visual Acuity (ETDRS)	X	X	X	X	X	X
Review of Qualification Criteria	X	X				
Slit-lamp Biomicroscopy	X	X	X	X	X	X
TFBUT	X	X		X		X
Fluorescein Staining - NEI Scale	X	X	X	X	X	X
Lissamine Green Staining - Oxford Scale	X	X		X		X
InflammaDry		X		X		X
Schirmer's Test I (without anesthesia)	X	X	X	X	X	X
Intraocular Pressure	X			X		X
Dilated Fundoscopy	X					X
Randomization		X				
In-office instillation of run-in	X					
Run-in & Diary Dispensation	X					
Run-in Collection		X				
In-office instillation of randomized study drug		X				
Drop Comfort Scale and Questionnaire		X				
Randomized Study Drug Dispensation		X	X	X	X	

Randomized Study Drug Collection			X	X	X	X
Diary review/collection		X	X	X	X	X
Study Exit						X

Source: Appendix 1 of CYS-003 Study Protocol

Table 15 Schedule of Visits and Measurements of Study CYS-004

Procedure	**Visit 0 Day -14 ± 2**	**Visit 1 Day 1**	**Visit 2 Day 15 ± 2**	**Visit 3 Day 29 ± 2**
Informed Consent / HIPAA	X			
Medical / Medication History and Demographics	X			
Review of Qualification Criteria	X	X		
Urine Pregnancy Test (as needed)	X			X
Medical/Medication Update		X	X	X
Recording of Adverse Events	X	X	X	X
Dryness Score (VAS)	X	X	X	X
VAS (frequency of dryness, awareness of dryness symptoms, blurred vision, reading problems, fluctuating vision, looking at screens, driving at night)	X	X	X	X
OSDI	X	X	X	X
Visual Acuity (ETDRS)	X	X	X	X
Slit-lamp Biomicroscopy	X	X	X	X
Tear Film Break-Up Time	X	X		X
Fluorescein Staining - NEI Scale	X	X	X	X
Lissamine Green Staining - Oxford Scale	X	X	X	X
Reading Assessments (MNRead, sustained silent reading, IReST)		X		X
Schirmer's Test I (without anaesthesia)	X	X		X
Intraocular Pressure	X			X
Dilated Fundoscopy	X			X
In-office instillation of run-in	X			
Run-in & Diary Dispensation	X			
Run-in Collection		X		
Randomization		X		
In-office instillation of randomized study drug		X	X	

Drop Comfort Scale and Questionnaire		X	X	
Eyedrop Acceptability Questionnaire				X
Dispensation of trial medication		X	X	
Collection of trial medication			X	X
Diary review/collection		X	X	X
Trial Exit				X

Source: Appendix 1 of CYS-004 Study Protocol

APPENDIX 2: Summary of Missing Data

Table 16 Summary of Subjects with Missing Values at Day 29

CYS-003			
	CyclASol (N=162)	Vehicle (N=166)	Total (N=328)
Subjects with missing at Day 29	5 (3.1 %)	1 (0.6 %)	6 (1.8 %)
Subjects who had missing at Day 29 but completed study	0	0	0
Subjects who discontinued study before Day 29 due to intercurrent events	5 (3.1 %)	1 (0.6 %)	6 (1.8 %)
Adverse Events	1	0	1
Administrative Reasons	1	1	2
Subject Choice	3	0	3
CYS-004			
	CyclASol (N=423)	Vehicle (N=411)	Total (N=834)
Subject with missing at Day 29	14 (3.3 %)	16 (3.9 %)	30 (3.6 %)
Subjects who completed study but had missing due to out of window visits greater than 2 days	6 (1.4 %)	7 (1.7 %)	13 (1.6 %)
Subjects who discontinued study	8 (1.9 %)	9 (2.2 %)	17 (2.0 %)
Adverse Events	2	0	2
Lost to Follow Up	0	3	3
Subject Choice	5	5	10
Other	1	1	2

Table 17 Summary of Subjects with Missing Values at Day 85

CYS-003			
	CyclASol (N=162)	Vehicle (N=166)	Total (N=328)
Subjects with missing at Day 85	7 (4.3%)	3 (1.8%)	10 (3.0%)
Subjects who had missing at Day 85 but completed study	0	0	0
Subjects who discontinued study before Day 85 due to intercurrent events	7 (4.3%)	3 (1.8%)	10 (3.0%)
Adverse Events	2	0	2
Administrative Reasons	2	2	4
Subject Choice	3	0	3
Other	0	1	1

APPENDIX 3: Sensitivity Analysis Results

Table 18 Sensitivity Analyses for Primary Sign Endpoint (Change from Baseline in tCFS score at Day 29)

		CYS-003		CYS-004	
		CyclASol 0.1%	**Vehicle**	**CyclASol 0.1%**	**Vehicle**
PPS	**LS Mean (SE)**	-3.50 (0.18)	-2.66 (0.17)	-3.95 (0.15)	-3.56 (0.15)
	LS Mean Difference (SE)	-0.84 (0.23)		-0.39 (0.19)	
	95% CI	(-1.30, -0.39)		(-0.75, -0.02)	
	p-value	0.0003		0.0366	
LOCF	**LS Mean (SE)**	-3.45 (0.17)	-2.64 (0.17)	-3.92 (0.14)	-3.48 (0.15)
	LS Mean Difference (SE)	-0.81 (0.22)		-0.43 (0.18)	
	95% CI	(-1.25, -0.37)		(-0.79, -0.07)	
	p-value	0.0003		0.0182	

The results are based on ANCOVA model with baseline value as covariate and site and treatment as factors.

Table 19 Sensitivity Analyses for Primary Symptom Endpoint of CYS-004 (Change from Baseline in Dryness Score at Day 29)

		CYS-003		CYS-004	
		CyclASol 0.1%	**Vehicle**	**CyclASol**	**Vehicle**
PPS	**LS Mean (SE)**	-10.6 (1.72)	-6.0 (1.67)	-11.9 (1.31)	-13.5 (1.33)
	LS Mean Difference (SE)	-4.6 (2.26)		1.6 (1.65)	
	95% CI	(-9.04, -0.15)		(-1.62, 4.86)	
	p-value	0.0430		0.3266	
LOCF	**LS Mean (SE)**	-10.4 (1.65)	-6.0 (1.62)	-12.0 (1.24)	-13.2 (1.27)
	LS Mean Difference (SE)	-4.5 (2.18)		1.2 (1.59)	
	95% CI	(-8.74, -0.17)		(-1.92, 4.33)	
	p-value	0.0418		0.4492	

The results are based on ANCOVA model with baseline value as covariate and site and treatment as factors.

Table 20 Sensitivity Analyses for Post-hoc Analysis Endpoint (Proportion of Schirmer's Tear Test Responders)

		CYS-003		CYS-004	
		CyclASol (N=162)	Vehicle (N=166)	CyclASol (N=423)	Vehicle (N=411)
PPS	**n**	152	161	404	391
	Responders, n (%)	19 (12.50 %)	9 (5.59 %)	44 (10.89 %)	27 (6.91 %)
	Difference (95% CI)[1]	6.91% (0.57%, 13.25%)		3.99% (0.04%, 7.93%)	
	p-value[1]	0.0323		0.0488	
	Odds Ratio (95% CI) [2]	2.41 (1.08, 5.77)		1.65 (1.00, 2.75)	
	p-value[2]	0.0367		0.0506	
	p-value[3]	0.0461		0.0615	
LOCF	**n**	162	166	423	411
	Responders, n (%)	19 (11.73 %)	9 (5.42 %)	44 (10.40 %)	27 (6.57 %)
	Difference (95% CI)[1]	6.31% (0.27%, 12.34%)		3.83% (0.06%, 7.60%)	
	p-value[1]	0.0410		0.0474	
	Odds Ratio (95% CI) [2]	2.32 (1.04, 5.53)		1.65 (1.01, 2.75)	
	p-value[2]	0.0458		0.0492	
	p-value[3]	0.0484		0.0621	

[1] The 95% CI and p-value are based on Normal approximation.
[2] The odds ratio, 95% CI and p-value are calculated using logistic regression model with a factor for treatment group.
[3] The p-value is based on Fisher's exact test.

APPENDIX 4: Efficacy Results at Other Visits (Time Trend)

(1) Change from Baseline in tCFS Score over Visits

Figure 4 Time Trend of Sign Endpoint

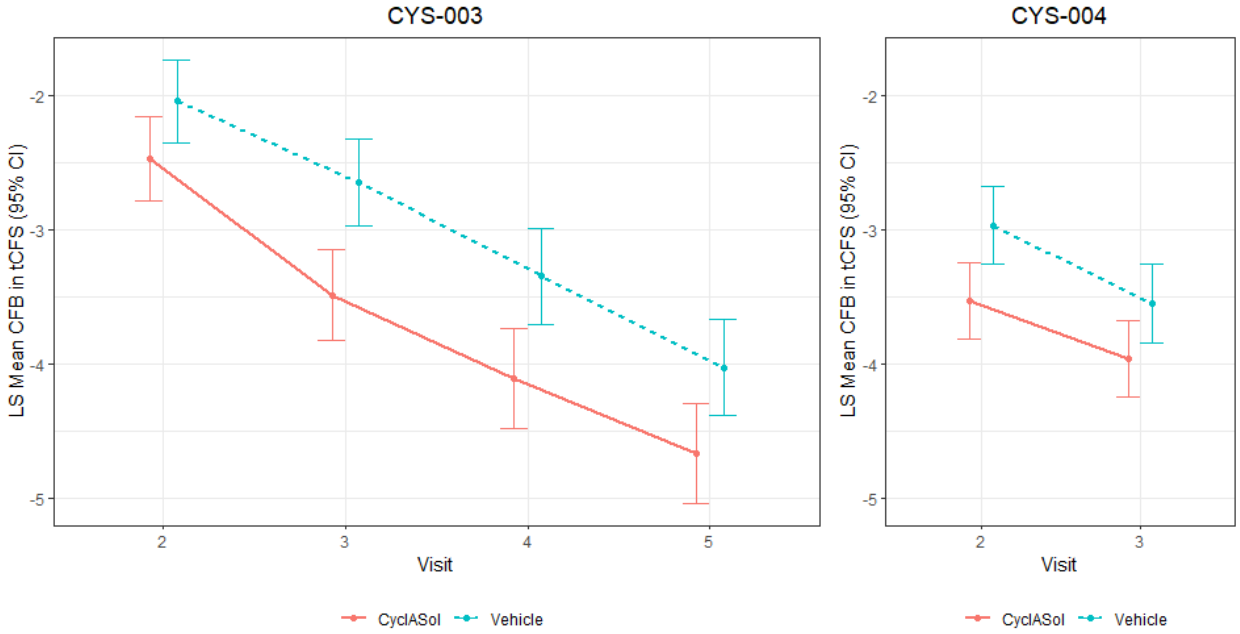


Table 21 Analysis Results for Change from Baseline in tCFS score at Visits Other than Day 29 (Visit 3) (Secondary Efficacy Endpoint; FAS with Available Data)

		CYS-003		CYS-004	
		CyclASol 0.1% (N=162)	Vehicle (N=166)	CyclASol 0.1% (N=423)	Vehicle (N=411)
Day 15 (Visit 2)	n	160	165	418	403
	LS Mean (SE)	-2.47 (0.160)	-2.04 (0.157)	-3.53 (0.144)	-2.968 (0.147)
	LS Mean Difference (SE)	-0.428 (0.211)		-0.562 (0.183)	
	95% CI	(-0.8436, -0.0128)		(-0.9217, -0.2023)	
	p-value	0.0434		0.0022	
Day 57 (Visit 4)	n	155	164		
	LS Mean (SE)	-4.11 (0.189)	-3.35 (0.183)		
	LS Mean Difference (SE)	-0.763 (0.249)			
	95% CI	(-1.2518, -0.2739)			
	p-value	0.0023			
Day 85 (Visit 5)	n	155	163		
	LS Mean (SE)	-4.67 (0.189)	-4.02 (0.183)		
	LS Mean Difference (SE)	-0.641 (0.249)			
	95% CI	(-1.1302, -0.1513)			
	p-value	0.0105			

The results are based on ANCOVA model with baseline value as covariate and site and treatment as factors.

(2) Change from Baseline in Total OSDI score over Visits

Figure 5 Time Trend of Symptom Endpoint of CYS-003 (CFB in Total OSDI Score)

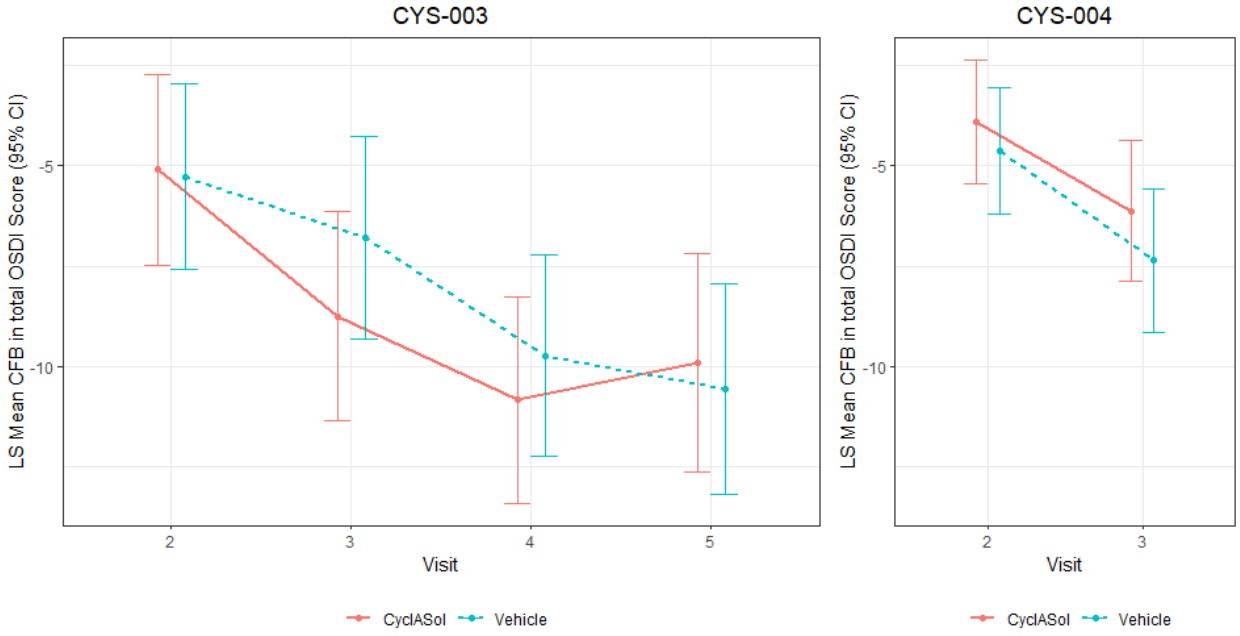


Table 22 Analysis Results for Change from Baseline in Total OSDI Score at Visits Other than Day 29 (Visit 3) (Secondary Efficacy Endpoint; FAS with Available Data)

		CYS-003		CYS-004	
		CyclASol 0.1% (N=162)	Vehicle (N=166)	CyclASol 0.1% (N=423)	Vehicle (N=411)
Day 15 (Visit 2)	n	160	165	418	403
	LS Mean (SE)	-5.10 (1.21)	-5.27 (1.18)	-3.90 (0.788)	-4.63 (0.804)
	LS Mean Difference (SE)	0.170 (1.59)		0.722 (1.01)	
	95% CI	(-2.9758, 3.2988)		(-1.2534, 2.6965)	
	p-value	0.9147		0.4735	
Day 57 (Visit 4)	n	156	164		
	LS Mean (SE)	-10.84 (1.31)	-9.73 (1.27)		
	LS Mean Difference (SE)	-1.11 (1.72)			
	95% CI	(-4.5005, 2.2871)			
	p-value	0.5216			
Day 85 (Visit 5)	n	155	163		
	LS Mean (SE)	-9.91 (1.38)	-10.58 (1.34)		
	LS Mean Difference (SE)	0.666 (1.82)			
	95% CI	(-2.9055, 4.2381)			
	p-value	0.7138			

The results are based on ANCOVA model with baseline value as covariate and site and treatment as factors.

(3) Change from Baseline in Dryness Score over Visits

Figure 6 Time Trend of Symptom Endpoint of CYS-004 (CFB in Dryness Score)

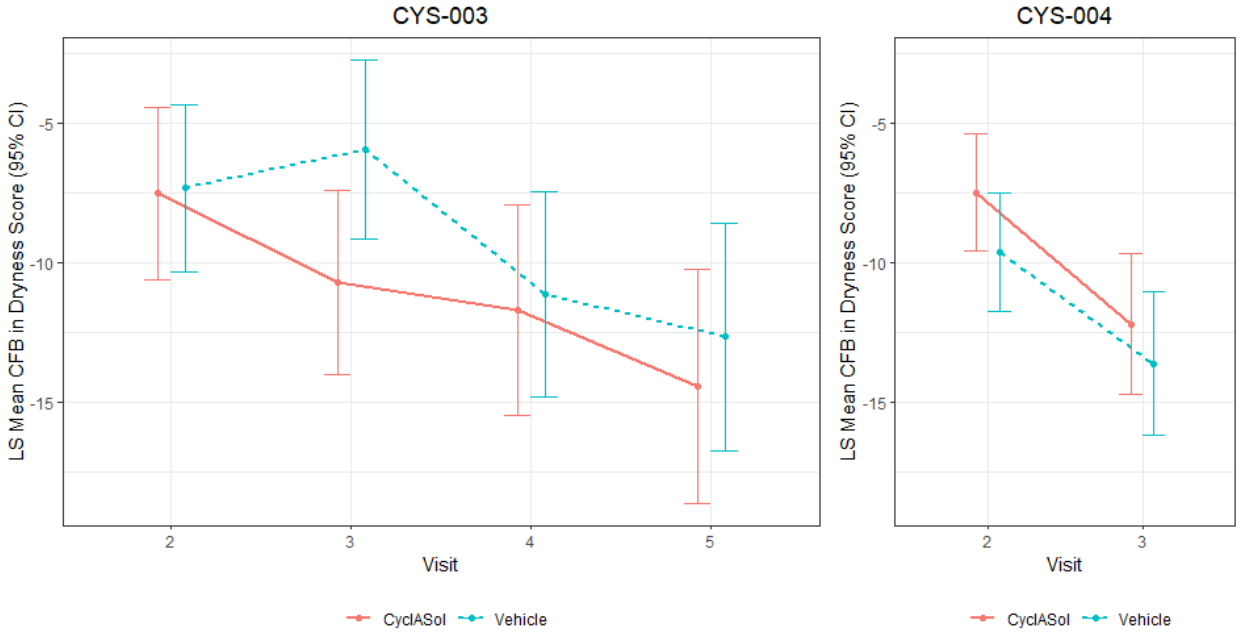


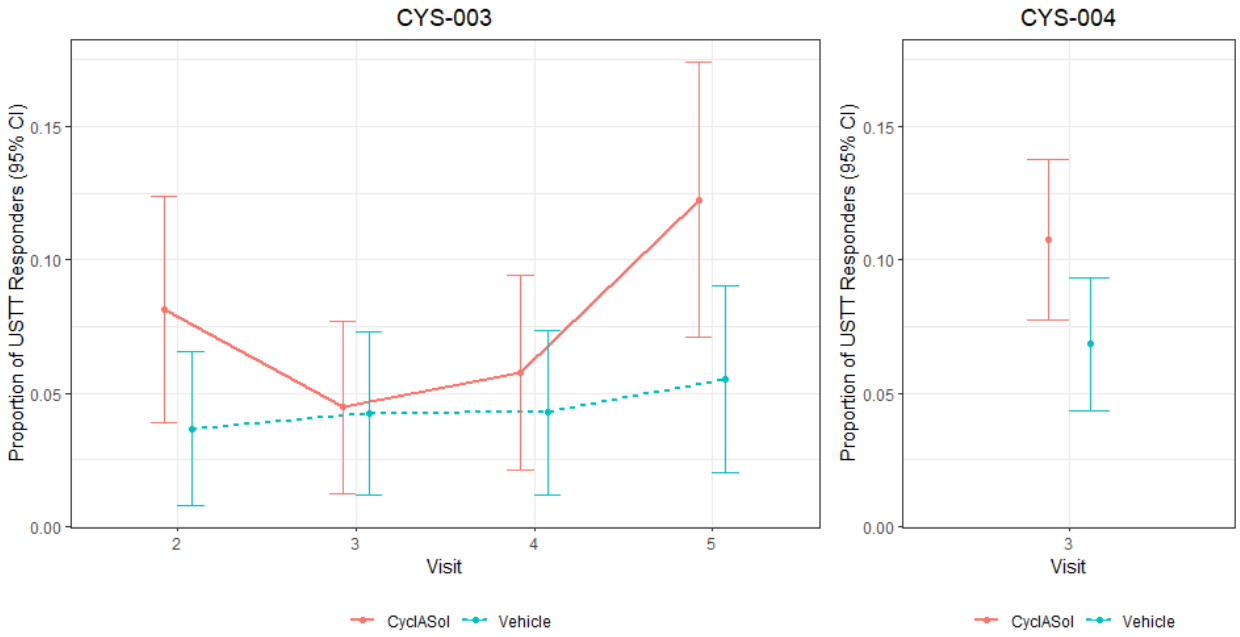
Table 23 Analysis Results for Change from Baseline in Dryness Score at Visits Other than Day 29 (Visit 3) (Secondary Efficacy Endpoint; FAS with Available Data)

		CYS-003		CYS-004	
		CyclASol 0.1% (N=162)	Vehicle (N=166)	CyclASol 0.1% (N=423)	Vehicle (N=411)
Day 15 (Visit 2)	n	160	165	418	403
	LS Mean (SE)	-7.51 (1.56)	-7.32 (1.53)	-7.48 (1.07)	-9.62 (1.09)
	LS Mean Difference (SE)	-0.197 (2.06)		2.15 (1.36)	
	95% CI	(-4.2509, 3.8564)		(-0.5261, 4.8221)	
	p-value	0.9238		0.1153	
Day 57 (Visit 4)	n	156	164		
	LS Mean (SE)	-11.7 (1.93)	-11.1 (1.87)		
	LS Mean Difference (SE)	-0.556 (2.54)			
	95% CI	(-5.5464, 4.4342)			
	p-value	0.8266			
Day 85 (Visit 5)	n	155	163		
	LS Mean (SE)	-14.4 (2.13)	-12.7 (2.07)		
	LS Mean Difference (SE)	-1.77 (2.81)			
	95% CI	(-7.2973, 3.7505)			
	p-value	0.5280			

The results are based on ANCOVA model with baseline value as covariate and site and treatment as factors.

(4) Proportion of Responders in Schirmer's Tear Test over Visits

Figure 7 Time Trend of Post-hoc Analysis Endpoint (Proportion of Schirmer's Tear Test Responders)



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Office of Clinical Pharmacology Review

NDA Number	217469
Link to EDR	\\CDSESUB1\evsprod\NDA217469\0001
Submission Date	8/8/2022
Submission Type	NDA 505(b)(2); Standard
Brand Name	Vevye
Generic Name	Cyclosporine
Dosage Form and Regimen	0.1% w/v ophthalmic solution administered as one drop twice a day in each eye approximately 12 hours apart.
Route of Administration	Ophthalmic
Proposed Indication	For the topical treatment of the signs and symptoms of dry eye disease
Applicant	Novaliq GmbH
Associated IND	IND (b) (4)
OCP Review Team	Amer Al-Khouja, Ph.D. M.H.S. Ping Ji, Ph.D.

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1 EXECUTIVE SUMMARY

Cyclosporine is a cyclic polypeptide calcineurin inhibitor that acts as an immunosuppressive agent when administered systemically. Oral and injectable formulations of cyclosporine have been approved for the prophylaxis of organ rejection in kidney, liver, and heart transplants; for the treatment of rheumatoid arthritis where the disease has not adequately responded to methotrexate; and for the treatment of severe, recalcitrant plaque psoriasis in patients who have failed to respond to at least one systemic therapy.

Cyclosporine has also been approved for ophthalmic use, as follows:

- Cyclosporine ophthalmic emulsion, 0.05% (Restasis) to increase tear production in patients whose tear production is presumed to be suppressed due to ocular inflammation associated with keratoconjunctivitis sicca (dry eye)
- Cyclosporine ophthalmic emulsion, 0.1% (Verkazia) for the treatment of vernal keratoconjunctivitis in children and adults
- Cyclosporine ophthalmic solution, 0.09% (Cequa) to increase tear production in patients with keratoconjunctivitis sicca (dry eye)

The Applicant, Novaliq GmbH, has submitted a 505(b)(2) NDA for an ophthalmic solution containing 0.1% w/v cyclosporine for the topical treatment of the signs and symptoms of dry eye disease (DED) (proposed trade name: Vevye). The proposed dosage regimen is to instill one drop twice a day in each eye approximately 12 hours apart.

Data from five clinical studies were submitted to support development of the proposed product. The studies include a phase 1 single- and multiple-dose PK and safety study in healthy subjects (CYS-001), a phase 2 multiple-dose efficacy, safety, and PK dose-ranging trial in subjects with DED (CYS-002), a phase 2b/3 efficacy and safety study in subjects with DED (CYS-003), and a phase 3 efficacy and safety study plus open-label extension in subjects with DED (CYS-004 and CYS-005).

The clinical pharmacology review focused on the PK of cyclosporine following administration of the proposed product, as assessed in studies CYS-001 and CYS-002.

1.1 Recommendations

The Office of Clinical Pharmacology has reviewed the clinical pharmacology data submitted in support of NDA 217469 and found the application acceptable to support approval from a clinical pharmacology perspective. The key review issues with specific clinical pharmacology recommendations and comments are summarized in **Table 1**.

Table 1. Summary of clinical pharmacology review issues and recommendations

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	Cyclosporine ophthalmic solution, 0.1% w/v is administered as an eye drop and is locally acting. As a

	result, systemic exposure does not impact the treatment effect. Clinical pharmacology information does not provide pivotal or supportive evidence of effectiveness.
General dosing instructions	One drop twice a day in each eye approximately 12 hours apart
Dosing in patient subgroups (intrinsic and extrinsic factors)	No dosage adjustment in any patient subgroups is needed
Labeling	Refer to Section 2.4
Bridge between the to-be-marketed and clinical trial formulations	The to-be-marketed formulation was used in the pivotal clinical studies.

1.2 Post-Marketing Requirement/Post-Marketing Commitment

None.

2 SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

The primary clinical pharmacology assessment included a review of cyclosporine exposure data from studies CYS-001 and CYS-002.

The PK of cyclosporine after ophthalmic administration has previously been evaluated for approved products (i.e., Cequa, Verkazia, Restasis). For all three products, blood concentrations of cyclosporine were either undetectable (i.e., below the lower limit of quantitation [LLOQ]) in all patients, or undetectable in a proportion of patients. Detectable concentrations were only marginally above the LLOQ.

2.1 Pharmacology and Clinical Pharmacokinetics

The PK of cyclosporine after ophthalmic administration was assessed in CYS-001 and CYS-002. Study CYS-001, conducted in healthy subjects, used a 0.05% w/v drug product formulation consisting of 0.5 mg/mL cyclosporine in perfluorobutylpentane (F4H5) with (b)(4)% w/w ethanol. Two different formulations were assessed in dose-ranging study CYS-002, which was conducted in subjects with dry eye disease. The formulations included a 0.05% w/v formulation with (b)(4)% w/w ethanol, and a 0.1% w/v formulation (1.0 mg/mL cyclosporine) with (b)(4)% w/w ethanol. The latter 0.1% formulation is the to-be-marketed formulation.

In studies CYS-001 and CYS-002, cyclosporine blood concentrations were below the LLOQ (0.100 ng/mL) in all samples at all timepoints. Ophthalmic administration of cyclosporine using the proposed product yielded no measurable systemic exposure. This is consistent with observed PK from approved ophthalmic cyclosporine products.

2.2 Dosing and Therapeutic Individualization

2.2.1 General Dosing

The recommended dosage is to instill one drop twice a day in each eye approximately 12 hours apart. The clinical efficacy and safety of this dosing regimen was assessed in studies CYS-003 and CYS-004 in adult patients with dry eye disease.

2.2.2 Therapeutic Individualization

The Applicant has not proposed any therapeutic individualization. The available clinical pharmacology information does not warrant a need for therapeutic individualization.

2.3 Outstanding Issues

None.

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 cyclosporine ophthalmic solution, 0.1% w/v (proposed trade name: Vevye) for the treatment of the signs and symptoms of dry eye disease. Cyclosporine is a cyclic polypeptide calcineurin inhibitor that acts as an immunosuppressive agent when administered systemically. In patients whose tear production is presumed to be suppressed due to ocular inflammation associated with dry eye disease, topical administration of cyclosporine is thought to act as a partial immunomodulator. However, the exact mechanism of action is not known.

The proposed product was evaluated under IND (b) (4). A summary of key clinical pharmacology-related discussions and correspondence with the Applicant are listed in Table 2 below.

Table 2. Summary of key clinical pharmacology-related communication with the Applicant

IND (b) (4), pre-IND (July 2015)	▪ The Agency agreed with the Applicant's plan to evaluate systemic exposure to cyclosporine in study CYS-002. The Agency also agreed that PK evaluations in CYS-001 and CYS-002 would be sufficient provided that no significant changes are made to the proposed dosage regimen (i.e., 0.05% or 0.1% BID), and no additional significant changes are made to the formulation. ▪ The Agency indicated that the Applicant did not provide sufficient information on the proposed use of (b) (4)

Source: Reviewer's summary based on meeting minutes located in DARRTS for IND (b) (4)

3.2 General Pharmacology and Pharmacokinetic Characteristics

3.2.1 Pharmacokinetics

Study CYS-001 was a phase 1 single- and multiple-dose PK and safety study conducted in healthy subjects. All subjects received the proposed cyclosporine ophthalmic solution or placebo in a crossover fashion. Subjects received one drop in one eye on the evening Day 1, then one drop in each eye in the morning and evening on Days 2 and 3. On Days 9 to 11, subjects received two drops in each eye in the morning and evening. The formulation of the proposed drug product contained 0.05% w/v cyclosporine and (b) (4)% w/w ethanol.

In the initial dosing period, PK samples were collected on Day 2 before the morning dose and within 30 minutes after the morning dose; on Day 3 within 15 minutes before the evening dose; and after the evening dose on Day 3 at minutes 5, 15, 30, and 60, and hours 4, 8, 12, 24, 48, and 72. Samples were collected at similar timepoints for the latter dosing period with samples

collected prior to the morning dose on Day 9; within 30 minutes after the morning dose on Day 9; within 15 minutes before the evening dose on Day 11; and after the evening dose on Day 11 at minutes 5, 15, 30, and 60, and hours 4, 8, 12, 24, 48, and 72.

In study CYS-001, all plasma concentrations of cyclosporine in all samples were below the LLOQ of 0.100 ng/mL.

Study CYS-002 was a phase 2 dose-ranging study to assess efficacy, safety, and tolerability in subjects with moderate to severe dry eye disease. In the 112-day randomized treatment period, subjects received the proposed drug product at a strength of 0.05% or 0.1%, or vehicle. An open-label comparator arm in which subjects received Restasis (0.05% cyclosporine ophthalmic emulsion) was also included. Subjects were instructed to instill one drop in each eye in the morning and in the evening. This is the same as the proposed dosage regimen. The formulations of the proposed drug product contained either 0.05% w/v or 0.1% w/v cyclosporine and (b)(4)% w/w ethanol. The latter formulation is the proposed to-be-marketed formulation.

PK samples were collected from a subset of subjects in each treatment arm (n = 55; 12 to 17 per arm). Samples were collected on Day 1 pre-dose, and post-dose at hours 0.5, 1, 2, and 4. An additional sample was collected on Day 113, at the 16-week follow-up visit (Visit 5).

In study CYS-002, all plasma concentrations of cyclosporine in all samples were below the LLOQ of 0.100 ng/mL.

3.2.2 Pharmacodynamics

The Applicant measured ocular surface HLA-DR expression in study CYS-002, and tear fluid levels of MMP-9 in studies CYS-002 and CYS-003. These PD markers are not clinically recognized endpoints for the treatment of dry eye disease. The relationship between changes in HLA-DR expression and MMP-9 levels and clinical efficacy in dry eye disease is not established. Overall, no definitive conclusions could be drawn from the data presented. For additional information, refer to Section 4.2.

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?

Cyclosporine ophthalmic solution, 0.1% w/v is administered as an eye drop, and the site of action is the eye. Therefore, the systematic exposure is not expected to impact the treatment effect, and, thus, the clinical pharmacology information does not provide pivotal or supportive evidence of effectiveness. PD measurements of HLA-DR expression (CYS-002) and MMP-9 levels (CYS-002 and CYS-003) were intended to provide supportive evidence of effectiveness.

However, these PD markers are not clinically recognized endpoints. The relationship between changes in HLA-DR and MMP-9 and clinical efficacy in dry eye disease has not been established.

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

Cyclosporine was not found to be systemically available after ophthalmic administration of the proposed product. This supports the systemic safety of the product when administered at the proposed dosage regimen of one drop in each eye twice daily. We also defer to the clinical review to determine the appropriateness of the proposed dosing regimen for the treatment of patients with dry eye disease.

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

Cyclosporine blood concentrations were undetectable after ophthalmic administration of the proposed product. Therefore, no alternative dosing regimen or management strategy is required for subpopulations based on intrinsic factors.

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

The proposed product is an ophthalmic solution that is administered topically. Therefore, there are no clinically relevant food-drug interactions. Relevant drug-drug interactions have been identified when cyclosporine is available systemically. However, the available PK data indicate that cyclosporine is not systemically available after ophthalmic administration of the proposed product. Thus, no clinically meaningful drug-drug interactions are expected.

4 APPENDICES

4.1 Bioanalytical Method Review

For studies CYS-001 and CYS-002 whole blood concentrations of cyclosporine were determined using method BPCYC2, "Bioanalytical Procedure for the Determination of Cyclosporine in Human Whole Blood (Potassium EDTA) using LC-MS/MS." In this method, cyclosporine and cyclosporine-d_4 (internal standard) are extracted from potassium EDTA (K_2EDTA) human whole blood via liquid-liquid extraction using methyl-t-butyl ether (MTBE) after acidification. Organic extracts are washed with an alkaline solution, evaporated, and reconstituted. Prepared samples are analyzed using liquid chromatography atmospheric pressure ionization tandem mass spectrometry (LC-API/MS/MS). Validation results are summarized in report ARCYC2:

Method Validation Report ARCYC2 – Method Validation Report for the Determination of Cyclosporine in Human Whole Blood (K_2EDTA) Using LC-API/MS/MS ((b) (4) (b) (4))

Table 3. Summary of Method Validation Report ARCYC2.

Bioanalytical method review summary	Method validation adequate to support results in studies CYS-001 and CYS-002		
Method description	Sample extraction from K_2EDTA whole blood via liquid-liquid extraction; separation and detection using liquid chromatography atmospheric pressure ionization tandem mass spectrometry (LC-API/MS/MS)		
Materials used for calibration curve, QCs, & concentration	Cyclosporine, Lot# IOF343 (b) (4)		
Validated assay range	Cyclosporine: 0.100 to 100 ng/mL in human whole blood		
Source & lot of internal standard reagents	Cyclosporine internal standard (cyclosporine-d_4), Lot# 3-UPA-106-3, (b) (4) (b) (4)		
Regression model & weighting	Linear regression with $1/x^2$ weighting		
Validation parameters	**Method validation summary**		**Acceptability**
Calibration curve performance during accuracy & precision from accepted validation runs	No of standard calibrators from LLOQ to ULOQ	8	Yes
	Cumulative accuracy (%bias) from LLOQ to ULOQ Cyclosporine	-5.0 to 3.1%	Yes
	Cumulative precision (%CV) from LLOQ to ULOQ Cyclosporine	≤ 5.3%	Yes
QCs performance during accuracy & precision from accepted validation runs	Cumulative accuracy (%bias) in 3 QCs Cyclosporine	-9.9 to -8.1%	Yes
	Inter-batch %CV Cyclosporine	≤ 3.1%	Yes
Selectivity & matrix effect	Blank human whole blood was evaluated from six different lots as well as from a pooled human whole blood lot. In all cases, no		Yes

	interference was observed at the retention times of cyclosporine and its internal standard. An assessment of six individual control matrix samples spiked with cyclosporine at the LLOQ demonstrated %Bias ranging from -14.6 to -0.7%, and a %CV of 5.6%, demonstrating no matrix effects.	
Extraction recovery	Across low, middle, and high QCs, the percent recovery ranged from 71.8 to 73.7% for cyclosporine, and 80.0% for internal standard. Recovery appeared consistent across runs (%CV ≤ 3.3%),	Yes
Interference & specificity	Samples at the ULOQ of cyclosporine did not produce a significant internal standard response (defined as > 5% of mean internal standard peak response of the internal standard blank). Samples containing only the internal standard did not product a significant cyclosporine response (defined as >25% of the mean LLOQ standard peak response).	Yes
Hemolysis effect	Not assessed	N/A
Lipemic effect	Not assessed	N/A
Dilution linearity	Accuracy and precision demonstrated for cyclosporine at 200 ng/mL at dilution factors of 1:10, 1:20, and 1:50 (%Bias -14.7 to -10.0%; % CV ≤ 5.6%)	Yes
Bench-top/process stability	Room temperature stability of cyclosporine in whole blood established for up to 20 hours (%Bias -8.0 to -3.3% and %CV ≤ 2.6%). Processed sample (extracts) stability established for up to 30 hours at room temperature (%Bias -12.9 to -7.7% and %CV ≤ 2.4%) and up to 70 hours under refrigeration (%Bias -10.5 to -8.4% and %CV ≤ 3.3%). Re-injection stability was established for up to 38 hours (%Bias -10.3 to -8.6% and %CV ≤ 2.6%). Extracts were stored under refrigeration conditions while awaiting re-injection.	Yes
Freeze-Thaw stability	Established for up to five freeze-thaw cycles at -20 °C (%Bias -9.3 to -6.3% and %CV ≤ 2.2%); and at -70 °C (%Bias -10.2 to -9.0% and %CV ≤ 3.5%).	Yes
Long-term storage	Established stability for cyclosporine in human whole blood at -20 °C for up to 182 days (%Bias -0.3 to 3.2% and %CV ≤ 0.5%); and at -70 °C for up to 182 days (%Bias -2.6 to -0.2% and %CV ≤ 2.0%).	Yes
Carry over	Carryover was found to be < 20% of LLOQ based on analysis of blank matrix samples immediately following the high calibration standards	Yes

In general, bioanalytical method validation was found to be acceptable. The Applicant did not assess the effects of hemolysis or lipemia. However, given that cyclosporine was consistently undetectable in all samples from all subjects, it is acceptable.

In-study assay performance in studies CYS-001 and CYS-002 was also found to be acceptable.

Table 4. In-study bioanalytical method performance in CYS-001.

Method performance in study CYS-001

Assay passing rate	• A total of 6 runs were performed in CYS-001 and all passed acceptance criteria	Yes
Standard curve performance	Performance of 8 standard calibrators: • Cumulative accuracy (% bias) range: -4.4 to 3.0% • Cumulative precision (% CV): ≤ 4.9%	Yes
QC performance	Performance of 3 QCs: • Cumulative accuracy (% bias) range: -1.6 to 1.7% • Cumulative precision (% CV): ≤ 6.7%	Yes
Method reproducibility	Incurred sample reanalysis was not conducted as cyclosporine concentrations in all samples were below the limit of quantitation.	Yes
Repeat analysis	• Bioanalytical report does not indicate that analysis was repeated for any samples.	Yes
Study sample analysis/ stability	Samples were stored at -70 °C until analysis. Samples were received by the analytical laboratory in May 2014 and extracted and analyzed in May 2014. The report specifies that study samples were stored a maximum of 61 days. Thus, all samples were analyzed within the established stability of 182 days at -70 °C.	

Table 5. In-study bioanalytical method performance in CYS-002.

Method performance in study CYS-002		
Assay passing rate	• A total of 9 runs were performed in study CYS-002 • 1 run was rejected due to failure of QCs to meet prespecified acceptance criteria	Yes
Standard curve performance	Performance of 8 standard calibrators: • Cumulative accuracy (% bias) range: -3.8 to 2.3% • Cumulative precision (% CV): ≤ 5.1%	Yes
QC performance	Performance of 3 QCs: • Cumulative accuracy (% bias) range: -4.3 to -1.4% • Cumulative precision (% CV): ≤ 7.3%	Yes
Method reproducibility	Incurred sample reanalysis was not conducted as cyclosporine concentrations in all samples were below the limit of quantitation.	Yes
Repeat analysis	• 2/326 (0.6%) individual subject samples were re-analyzed ○ Both re-assayed samples were originally rejected due to low internal standard response (outside internal standard acceptance criteria).	Yes
Study sample analysis/ stability	Samples were stored at -70 °C until analysis. Samples were received by the analytical laboratory between Feb. and Aug. 2016. Per the report all samples were analyzed by Aug. 2016 and study samples were stored a maximum of 61 days. Thus, all samples were analyzed within the established stability of 182 days at -70 °C.	

4.2 Pharmacodynamics

In study CYS-002, the Applicant measured ocular surface HLA-DR as a market to support the anti-inflammatory activity of cyclosporine. Conjunctival impressions were collected at Baseline on Day 1, and on Day 113 (Visit 5). After sample processing, HLA-DR expression is measured via flow cytometry. The data shown in Table 6 are reported in arbitrary units of fluorescence.

Table 6. Fluorescence quantification of HLA-DR from conjunctival impressions in study CYS-002.

		CyclASol 0.05% (N=51)	CyclASol 0.1% (N=51)	Restasis (N=53)	Vehicle (N=52)
Fluorescence Quantification – All Samples					
Visit 1 (Baseline)	n	51	50	52	52
	Mean (SD)	46836.39 (50342.70)	41015.04 (34027.04)	55847.79 (47595.38)	38412.40 (20194.90)
Visit 5 (Day 113)	n	50	50	52	48
	Mean (SD)	34658.16 (23741.77)	32518.80 (16701.47)	37589.27 (26084.02)	36870.19 (19486.12)
	n	50	49	51	48
	CFB Mean (SD)	-11664.7 (39054.42)	-8833.49 (23916.49)	-18965.3 (40688.65)	-2211.67 (17064.82)

Note: "CyclASol" refers to the proposed drug product.
Source: Clinical Study Report for Study CYS-002

The data in Table 6 suggest that HLA-DR expression decreases in all treatment arms relative to placebo following 112 days of treatment at the proposed dosage regimen. However, there is high variability in all measurements, as indicated by the large standard deviations. Thus, no definitive conclusions can be made.

Changes in tear fluid levels of MMP-9, an inflammatory marker, was also assessed in studies CYS-002 and CYS-003. In study CYS-002, change from baseline in MMP-9 levels in tear fluid at Day 113 was assessed. In study CYS-003, a phase 2b/3 efficacy and safety study conducted in subjects with dry eye disease, change from baseline in MMP-9 levels at Days 29 and 85 was evaluated. The Applicant used the InflammaDry® test designed for in-office use. The test produces a binary readout based on an MMP-9 cutoff value of 40 ng/mL. A positive result indicates MMP-9 levels are ≥ 40 ng/mL, while a negative result indicates MMP-9 levels are < 40 ng/mL. Data for studies CYS-002 and CYS-003 are shown in Table 7 and Table 8, respectively.

Table 7. Proportion of patients with MMP-9 levels of 40 ng/mL or greater over time in study CYS-002.

		CyclASol 0.05% (N=51)	CyclASol 0.1% (N=51)	Restasis (N=53)	Vehicle (N=52)
Visit 1 (Baseline)	Positive	27 (52.9%)	18 (35.3%)	32 (60.4%)	26 (50.0%)
	Negative	24 (47.1%)	33 (64.7%)	21 (39.6%)	26 (50.0%)
Visit 5 (Day 113)	Positive	19 (38.0%)	9 (18.0%)	9 (17.3%)	8 (16.7%)
	Negative	31 (62.0%)	41 (82.0%)	43 (82.7%)	40 (83.3%)
	Odds Ratio, Visit 5:Baseline	0.55	0.40	0.14	0.20
	Difference in log (Odds), p-value	0.1057	0.0690	<0.0001	0.0012

Note: N in the headers represents the total number of subjects in each respective treatment group within the FAS population.
Sources: **Table 14.2.13.1; Appendix 16.2.6.15**

Note: "CyclASol" refers to the proposed drug product.
Source: Clinical Study Report for Study CYS-002

Table 8. Proportion of patients with MMP-9 levels of 40 ng/mL or greater over time in study CYS-003.

		CyclASol 0.1% (N=162)	Vehicle (N=166)
Visit 1 (Day 1, Baseline)	n	162	166
	Positive	71 (43.8%)	72 (43.4%)
	Negative	91 (56.2%)	94 (56.6%)
Visit 3 (Day 29)	n	157	163
	Positive	53 (33.8%)	70 (42.9%)
	Negative	104 (66.2%)	93 (57.1%)
	Odds Ratio, Visit 3:Baseline	0.6	1.0
	Two-sided 95% CI	(0.4, 0.9)	(0.7, 1.4)
	Difference in log (Odds), *P*-value	0.0171	0.8110
Visit 5 (Day 85)	n	155	158
	Positive	66 (42.6%)	77 (48.7%)
	Negative	89 (57.4%)	81 (51.3%)
	Odds Ratio, Visit 5:Baseline	0.9	1.2
	Two-sided 95% CI	(0.6, 1.5)	(0.8, 1.9)
	Difference in log (Odds), *P*-value	0.7868	0.3640

Abbreviations: CI = confidence interval; FAS = Full Analysis Set, GEE = generalized estimating equations

Note: N in headers represents the total number of subjects enrolled in each respective treatment group within the FAS population. Odds ratio estimates, confidence intervals and p-value for the difference in the log odds are calculated using a GEE model with treatment group, visit, and interaction of treatment group and visit as covariates, subjects as random effects, and an unstructured covariance matrix.

Sources: Tables 14.2.3.22; Listing 16.2.6.10

Note: "CyclASol" refers to the proposed drug product.
Source: Clinical Study Report for Study CYS-003

In study CYS-002, the proportion of patients with MMP-9 levels ≥ 40 ng/mL decreased in all treatment arms after 112 days of treatment. However, the largest changes occurred in the vehicle and Restasis arms. Meanwhile, in study CYS-003, the proportion of patients with MMP-9 levels ≥ 40 ng/mL appeared to decrease relative to baseline in patients randomized to receive cyclosporine on Day 29, but not on Day 85. The clinical meaningfulness of a cutoff of 40 ng/mL for MMP-9 concentrations is not clear. Nevertheless, given the conflicting results observed across the studies, no definitive conclusions can be drawn from this data.

The relationship between changes in HLA-DR expression and MMP-9 levels and clinical efficacy in dry eye disease is not established. Overall, no definitive conclusions can be made from the PD data presented.

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