Clinical Trial Protocol: CYS-007

Protocol Title:	A multi-center, randomized, double-masked, controlled clinical trial to evaluate the corneal endothelial health of dry eye disease subjects treated with cyclosporine ophthalmic solution, 0.1%
Protocol Number:	CYS-007
Trial Phase:	4
Investigational Product Name:	Cyclosporine ophthalmic solution, 0.1% (development name: CyclASol, US brand name VEVYE)
IND Number:	128163
Indication:	Dry Eye Disease (*keratoconjunctivitis sicca*)
Sponsor:	Novaliq GmbH Im Neuenheimer Feld 515 69120 Heidelberg Germany
Contract Research Organization:	[redacted]
Original Protocol:	18 December 2023
Amendment 1:	21 February 2024

Confidentiality Statement

This protocol contains confidential, proprietary information of CRO and Novaliq GmbH. Further dissemination, distribution, or copying of this protocol or its contents is strictly prohibited.

Regulatory Statement

This trial will be performed in compliance with the protocol and in accordance with Good Clinical Practice (International Conference on Harmonisation [ICH], Guidance E6), principles of human subject protection, and applicable country-specific regulatory requirements.

1 SYNOPSIS AND TRIAL CONTACT INFORMATION

1.1 TRIAL CONTACT INFORMATION

SPONSOR PERSONNEL

Scientific Lead:	[redacted]
Director Clinical Development:	[redacted]
Manager Clinical Operations:	[redacted]

MEDICAL MONITOR

Medical Monitor	[redacted]

1.2 SYNOPSIS

Protocol Title:	A multi-center, randomized, double-masked, controlled clinical trial to evaluate the corneal endothelial health of dry eye disease subjects treated with cyclosporine ophthalmic solution, 0.1%
Protocol Number:	CYS-007
Investigational Products:	1) Cyclosporine ophthalmic solution, 0.1% (development name: CyclASol, US brand name VEVYE) 2) Saline solution, 0.6%
Trial Phase:	4
Trial Objectives:	The objective of this trial is to evaluate the effect of cyclosporine ophthalmic solution, 0.1% on corneal endothelial cell health in comparison to a hypotonic saline solution in subjects with Dry Eye Disease (DED)
Overall Trial Design	
Structure:	Multi-center, randomized, double-masked
Duration:	An individual subject's participation is approximately 52 weeks (12 months). The estimated trial duration from first subject first visit to last subject last visit is 18 months.
Dosage/Dose Regimen:	Eligible subjects will be randomized in a 2:1 ratio to one of the following treatments to be administered BID bilaterally from Visit 1 to Visit 5: • Cyclosporine ophthalmic solution, 0.1% • Saline solution, 0.6%
Summary of Visit Schedule:	5 onsite visits over the course of approximately 52 weeks • Visit 1 = Day 1, randomization and start of treatment • Visit 2 = week 12 follow-up ± 5 days, • Visit 3 = week 26 follow-up ± 5 days, • Visit 4 = week 40 follow-up ± 5 days, • Visit 5 = week 52 follow-up ± 5 days / trial exit 8 telephone calls (TC) • TC 1= week 4 follow-up ± 7 days • TC 2 = week 8 follow-up ± 7 days • TC 3 = week 16 follow-up ± 7 days • TC 4 = week 21 follow-up ± 7 days • TC 5 = week 30 follow-up ± 7 days • TC 6 = week 36 follow-up ± 7 days • TC 7 = week 44 follow-up ± 7 days • TC 8 = week 48 follow-up ± 7 days

Measures to reduce bias:	This is a randomized treatment assignment, double-masked, multi-center trial. Subjects will be instructed not to show or discuss the properties of the assigned Investigational Medicinal Product (IMP) and/or their experience with the IMP with other trial participants, and not to show or discuss the IMP with the Investigator or site staff [redacted] unless instructed to do so. [redacted]
Study Population Characteristics	
Number of Subjects:	Approximately 200 subjects will be enrolled and randomized in a 2:1 (cyclosporine ophthalmic solution, 0.1%: saline solution) fashion at approximately 6 sites to achieve at least 100 subjects treated with cyclosporine ophthalmic solution, 0.1% completing 52 weeks dosing with respective follow-up visits.
Condition/Disease:	Dry Eye Disease (*keratoconjunctivitis sicca*)
Inclusion Criteria:	**Subjects must:** 1. Be at least 18 years of age; 2. Provide written informed consent; 3. Have a subject reported history of dry eye disease (DED) [redacted] 4. Have an OSDI Score [redacted] 5. [redacted] 6. Have a baseline corneal endothelial cell density [redacted] 7. Be able and willing to follow instructions, including participation in all trial assessments and visits.
Exclusion Criteria:	**Subjects must not:** 1. Have any clinically significant slit-lamp findings or ocular conditions at Visit 1 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; 2. [redacted]

	3. Have a history of chronic or recurrent inflammatory eye disease (e.g., iritis, scleritis, uveitis, iridocyclitis, rubeosis iridis); 4. [redacted] 5. Have abnormal lid anatomy (e.g. incomplete eyelid closure, entropion, or ectropion) or abnormal blinking; 6. [redacted] 8. Have glaucoma or ocular hypertension (IOP > 20 mmHg) or have received a microinvasive glaucoma surgery (MIGS); 9. [redacted] 11. Be diagnosed with an ongoing ocular infection; 12. [redacted] 17. Have had intraocular surgery or ocular laser surgery or significant trauma within 365 days before Visit 1, or have any planned ocular surgeries during the trial period; 18. Have an uncontrolled systemic disease such as uncontrolled diabetes, severe renal insufficiency, progressive autoimmune diseases;

	[redacted] 20. Be a woman who is pregnant, nursing, or planning a pregnancy; 21. Be a woman of childbearing potential who is not using an acceptable means of birth control; acceptable methods of contraception include: hormonal – oral, implantable, injectable, or transdermal contraceptives; mechanical – spermicide in conjunction with a barrier such as a diaphragm or condom; intrauterine device (IUD); or surgical sterilization of 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. 22. Be currently using an investigational drug or device or have used an investigational drug or device within [redacted] 23. Have a condition or be in a situation (e.g. language barrier, non-cooperative, history of substance abuse) 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. 24. Have previously participated in CYS-002, CYS-003, and/or CYS-004 CyclASol/VEVYE clinical trials 25. Live in the same household with another randomized CYS-007 participant.
Trial Formulation:	• Cyclosporine ophthalmic solution, 0.1% (Cyclosporine A 0.1% in perfluorobutylpentane) • Saline solution, 0.6% (Sodium chloride solution 0.6% [redacted]
Evaluation Criteria	
Endpoint(s):	**Primary Safety Endpoint:** Change from baseline (CFB) in corneal endothelial cell density [ECD (count/mm²)] using specular microscopy at 12 months (Visit 5). **Secondary Safety Endpoints:** • ECD at month 3 and 12 and CFB at 3 months (Visit 2) • Endothelial cell coefficient of variation [CV] (polymegethism) and CFB at each measured visit

	• Endothelial cell hexagonality in % and CFB at each measured visit • Central corneal thickness and CFB at each measured visit • Ocular and non-ocular adverse events • Visual acuity and CFB at each measured post-baseline visit • Slit-lamp biomicroscopy at each measured post-baseline visit • Intraocular pressure (IOP) and CFB at each measured post-baseline visit • Dilated fundoscopy at each measured post-baseline visit
[redacted]	• [redacted]

[redacted] Statistical Methods and Types of Analyses

Sample Size:
Approximately 200 subjects will be enrolled and randomized in a 2:1 fashion (cyclosporine ophthalmic solution, 0.1%: saline solution, 0.6%) at approximately 6 sites to achieve at least 100 subjects treated with cyclosporine ophthalmic solution, 0.1% completing 52 weeks dosing with respective follow-up visits.

Primary Safety Analyses:
ECD (cells/mm^2) and change from baseline in the study eye will be summarized by treatment group and follow-up visit using the sample size, mean, standard deviation, median, minimum, and maximum. Change from baseline in ECD at Visit 5 will be analyzed using an ANCOVA model with treatment group as a fixed effect, and baseline value as a covariate. Treatment group estimates and LS means will be presented from the model, along with 95% confidence intervals. The primary analysis will summarize observed data only.

Secondary Safety Analyses:
Secondary analyses will be based on observed data only. All safety endpoints captured using specular microscopy will be analyzed and summarized using the same descriptive methodology as the primary analysis. This includes both study eye and fellow eye.

All other visit-based safety data will be analyzed by visit using change or shift from baseline, where applicable. Assessments performed by eye (study eye and fellow eye) will be summarized separately. Shifts from baseline to on-treatment value for ocular safety assessments will be summarized.

Adverse events (AEs) will be coded using the MedDRA dictionary. Frequencies and percentages of subjects with treatment-emergent adverse events (TEAEs), serious TEAEs, and TEAEs causing premature discontinuation will be provided by treatment arm and visit. Separate summaries will be performed for ocular and non-ocular AEs.

TABLE OF CONTENTS

Clinical Trial Protocol: CYS-007 1
1 SYNOPSIS AND TRIAL CONTACT INFORMATION 2
1.1 TRIAL CONTACT INFORMATION 2
1.2 SYNOPSIS 3
TABLE OF CONTENTS 8
TABLE OF FIGURES 10
LIST OF ABBREVIATIONS 11
2 INTRODUCTION 12
2.1 Dry Eye Disease (DED) 12
2.2 Product 12
2.3 Trial Rational 13
2.4 Summary of Known and Potential Risks and Benefits to Human Subjects 13
3 TRIAL OBJECTIVES 15
3.1 Primary objective 15
3.2 Secondary objective 15
4 TRIAL DESIGN 15
4.1 Overall Trial Design 15
4.2 End of Trial Definition 15
4.3 Visit Description 15
4.4 Trial Flow 17
4.5 Enrollment and Treatment Assignment 17
4.6 Justification of Trial Design 17
4.7 Justification for Dose 17
5 TRIAL POPULATION 18
5.1 Number of Subjects (approximate) 18
5.2 Trial Population Characteristics 18
5.3 Inclusion Criteria 18
5.4 Exclusion Criteria 18
[redacted]
5.6 Subject/ Trial Withdrawal Criteria 20
6 TRIAL PARAMETERS 21
6.1 Safety Endpoints 21
6.1.1 Primary Safety Endpoint 21
6.1.2 Secondary Safety Endpoints: 21
[redacted]
7 TRIAL MATERIALS 22
7.1 Investigational Medicinal Product 22
7.1.1 IMP/ Formulation 22

7.1.2 Labeling and Packaging of IMP 22
7.1.3 IMP Storage 23
7.1.4 IMP Dispensation....23
7.1.5 Instructions for Use and Administration....24
7.2 IMP Accountability 24
7.3 IMP Handling and Disposal 24
7.4 Other Trial Supplies....24
8 TRIAL METHODS AND PROCEDURES 25
8.1 Concurrent Medications and Therapies....25
8.2 Prohibited Treatments 25
8.3 Restrictions and Prohibitions 26
8.4 Examination Procedures....26
8.4.1 Procedures to be Performed at Each Trial Visit with Regard to Trial Objective(s)....26
8.4.2 Early Termination/Discontinuations....28
8.4.3 Unscheduled Visits 28
8.5 Endothelial Cell Density Assessment and Analysis 29
8.6 Compliance with Dosing/Protocol Deviations....29
8.7 Subject Disposition 29
8.7.1 Completed Subjects 29
8.7.2 Discontinued Subjects....30
8.8 Trial Termination....30
8.9 Trial Duration....30
9 ADVERSE EVENTS 31
9.1 Adverse Event....31
9.1.1 Severity 31
9.1.2 Relationship to Investigational Product....32
9.1.3 Expectedness....32
9.1.4 Outcome....33
9.1.5 Period of Observation 33
9.2 Serious Adverse Events....33
9.3 Procedures for Reporting Adverse Events....34
9.3.1 Reporting a Serious Adverse Event 34
9.3.2 Reporting a Suspected Unexpected Serious Adverse Reaction....34
9.4 Procedures for Unmasking of IMP....35
9.5 Type and Duration of the Follow-up of Subjects after Adverse Events....35
9.6 Procedures for Reporting Pregnancies....36
10 STATISTICAL HYPOTHESES AND METHODS OF ANALYSES....37
10.1 Analysis Populations 37
10.2 Statistical Hypotheses....37
10.3 Sample Size 37

10.4 Statistical Analysis....37
10.4.1 General Considerations....37
10.4.2 Unit of Analysis....37
10.4.3 Missing Data....38
10.4.4 Primary Safety Analyses....38
10.4.5 Secondary Safety Analyses....38
[redacted]
11 COMPLIANCE WITH GOOD CLINICAL PRACTICES, ETHICAL CONSIDERATIONS, AND ADMINISTRATIVE ISSUES....39
11.1 Protection of Human Subjects....39
11.1.1 Subject Informed Consent....39
11.1.2 Institutional Review Board (IRB) Approval....39
11.2 Ethical Conduct of the Trial....39
11.3 Subject Confidentiality....40
11.4 Documentation....40
11.4.1 Retention of Documentation....40
11.5 Recording of Data on Source Documents and Electronic Case Reports Forms (eCRFs)....40
11.6 Monitoring and Quality Assurance....41
11.7 Handling of Biological Samples....41
11.8 Publications....41
12 REFERENCES....42
Appendix 1: Schedule of Assessments....44
Appendix 2: Examination Procedures, Tests, Equipment, and Techniques....45
Appendix 3: Protocol Amendment 1 Summary....58
Appendix 4: Change History....60
Appendix 5: Sponsor and CRO Approvals....61
Appendix 6: Investigator's Signature....62

TABLE OF FIGURES

Figure 1: Mean change (SD) from baseline and treatment difference (cyclosporine ophthalmic solution, 0.1% – vehicle) in tCFS score in subjects with DED.... 14
Figure 2: Trial Flow.... 17

LIST OF ABBREVIATIONS

AE	Adverse Event
BID	Twice Daily
CFB	Change from Baseline
CFR	Code of Federal Regulations
[redacted]	[redacted]
eCRF	Electronic Case Report Form
CRO	Contract Research Organization
CV	Endothelial cell coefficient of variation
DED	Dry Eye Disease
DEWS	(International) Dry Eye Workshop
ECD	Endothelial cell density
ET	Early termination
ETDRS	Early Treatment of Diabetic Retinopathy Study
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Information Portability and Accountability Act
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IND	Investigational New Drug Application
IOP	Intraocular Pressure
IRB	Independent Review Board
IRT	Interactive Response Technology
logMAR	Logarithm of the Minimum Angle of Resolution
MedDRA	Medical Dictionary for Regulatory Activities
ml	Milliliter
µl	Microliter
mm	Millimeter
mmHg	Millimeters of Mercury
NEI	National Eye Institute
OD	Right Eye
OS	Left Eye
OSDI	Ocular Surface Disease Index
SAE	Serious Adverse Event
SAF	Safety Set
[redacted]	[redacted]
TEAE	Treatment-emergent Adverse Event
US	United States
VA	Visual Acuity
VAS	Visual Analog Scale
WHO	World Health Organization
w/v; w/w	Weight per volume / Weight per weight

2 INTRODUCTION

2.1 Dry Eye Disease (DED)

Dry Eye Disease (DED) is defined by the international Dry Eye Workshop II (DEWS II) as "a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles" (Craig et al. 2017). Symptoms of DED such as feeling of dryness, burning, a sandy/gritty sensation, foreign body sensation, pain or itchiness are quite debilitating. In addition, visual function related symptoms such as fluctuating vision with blinking, blurred vision, and difficulty with reading despite perfect visual acuity is an important and underestimated aspect of the disease. In consequence, DED negatively impacts quality of life comparably to other severe diseases (Schiffman et al. 2003), and adverse effects on mental health, such as depression and anxiety, have been observed (Le et al. 2012). DED is a serious disorder that, if left untreated or undertreated, progressively damages the ocular surface and may lead to vision loss due to corneal complications (Lemp et al. 1995).

As many as 5 - 35% of patients visiting ophthalmic clinics report DED symptoms, making it one of the most common conditions seen by ophthalmic specialists (McCarty et al. 1998; Lin et al. 2003). Estimates of the prevalence of DED vary considerably, depending on the criteria used to define the disease, but in the United States (US), it has been estimated that as many as 3.2 million women and 1.7 million men over the age of 50 have DED, with a projected 40% increase in the number of patients affected by 2030 (Schaumberg et al. 2002; Schaumberg et al. 2003; Schaumberg et al. 2009) With the aging population in the US and other countries of the developed world, and with increasing use of visual displays / computers, DED is expected to continue to become more prevalent and finding a treatment is becoming more important (Benitez-del-Castillo et al. 2017).

2.2 Product

The investigational product of this trial, cyclosporine ophthalmic solution, 0.1% has been approved for the treatment of signs and symptoms of DED by the US Food and Drug Administration (FDA) under its brand name VEVYE in May 2023 (VEVYE label information 2023).

The active ingredient, cyclosporine, is a calcineurin inhibitor, which has anti-inflammatory and selective immunomodulatory properties. Due to this mode of action, it has been widely studied as topical treatment for T-cell mediated diseases of the ocular surface such as DED.

Cyclosporine cannot be solubilized in the commonly used aqueous ophthalmic vehicles because of its hydrophobicity and extremely low aqueous solubility. Up to now, the only topical formulations of cyclosporine on the market are ophthalmic emulsions/nano-emulsions. Emulsions contain excipients, oils and surfactants often poorly tolerated and have a low bioavailability because cyclosporine is nonpolar and thus has a strong tendency to remain in the micelle (Lallemand et al. 2017).

In contrast, in VEVYE cyclosporine is dissolved in the novel excipient perfluorobutylpentane (F4H5). The novel excipient F4H5 is a lipophilic organic solvent and provides an improved bioavailability of cyclosporine as (1) cyclosporine is soluble in this lipophilic vehicle, (2) the vehicle has a significantly longer retention time on the ocular surface compared to a water-based eye drop and (3) due to its physicochemical properties, the vehicle spreads rapidly on the ocular surface after administration. [redacted] Moreover, the physical properties of the vehicle are ocular surface friendly and thereby potentially contribute to the efficacy of the product.

2.3 Trial Rational

The corneal endothelium is composed of a single layer of flat, polygonal cells, which line the posterior surface of the cornea. It is responsible for nourishing the cornea and maintaining proper hydration of the cornea through its barrier and pump functions. Loss of this function results in an inability for the cornea to properly respond to edematous stimuli and can ultimately lead to corneal decompensation.
These cells lie dormant in the G1 phase of the cell cycle, and there is no evidence of mitotic activity under normal conditions. Therefore, the corneal endothelial cell layer cannot regenerate, and the number of cells is reduced with aging. In adults, the mean endothelial cell density (ECD) ranges between 2000 and 3500 cells/mm^2 and decreases at a steady 0.6% per year (Kaur et al. 2023).
When there is endothelial injury, the healing is driven by cellular elongation and spread of residual cells, which also leads to a reduction in ECD (Kaur et al. 2023).
Ocular and systemic diseases can also impact corneal endothelial cells. Glaucoma is generally found to decrease ECD, whereas diabetes is associated with a reduced percentage of hexagonal cells but not with a reduction in ECD. There is little evidence that topical ocular medications affect the corneal endothelium (Moshirfar 2019).

This trial is designed to evaluate the corneal ECD (cells/mm²) of DED subjects treated with cyclosporine ophthalmic solution, 0.1% using specular microscopy at baseline and over a period of one year as determined by FDA as post-market requirement related to the approval of VEVYE to identify an unexpected serious risk of corneal endothelial cell loss.

2.4 Summary of Known and Potential Risks and Benefits to Human Subjects

The safety and efficacy of cyclosporine ophthalmic solution, 0.1% for the US approval were assessed in a total of 1369 subjects with DED, of which 738 received active treatment.

The program included three multi-center, randomized, adequate and well-controlled clinical trials in subjects who had DED (CYS-002, CYS-003 [ESSENCE-1] and CYS-004 [ESSENCE-2]) comparing cyclosporine ophthalmic solution, 0.1% with vehicle (Wirta et. al 2019; Sheppard et al. 2021; Akpek et al. 2023).

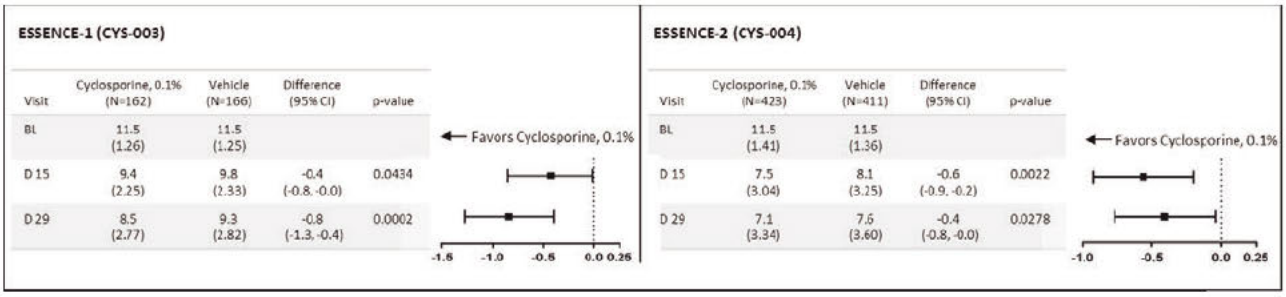
Effect on tear production

At Day 29 of CYS-002 and CYS-004, there was a statistically significantly higher percentage of eyes with an increase of ≥ 10 mm from baseline in Schirmer's test wetting. [redacted]

Effect on the ocular surface

At Days 15 and 29 on CYS-003 and CYS-004, a statistically significant reduction in total corneal fluorescein staining (tCFS) score favoring cyclosporine ophthalmic solution, 0.1% was observed in both studies (see Figure 1).

Figure 1: Mean change (SD) from baseline and treatment difference (cyclosporine ophthalmic solution, 0.1% – vehicle) in tCFS score in subjects with DED



In ESSENCE-1, a statistically significant improvement favoring cyclosporine ophthalmic solution, 0.1% was also observed at Days 57 and 85.

Responder analyses showed that the proportion of subjects with a clinically meaningful tCFS improvement of ≥ 3 grades at Day 29 was statistically significantly different and favoring cyclosporine ophthalmic solution, 0.1% in both studies at Day 29.

During the clinical program with 738 subjects receiving at least 1 dose of VEVYE, the most common adverse reactions were instillation site reactions (8%) and temporary decreases in visual acuity (3%).

In the clinical program no deaths were reported. [redacted] subjects reported treatment emergent SAEs. All were assessed as unrelated to study drug.

Ophthalmologic examinations of visual acuity, slit-lamp biomicroscopy, IOP, and dilated fundoscopy showed no meaningful differences between cyclosporine ophthalmic solution, 0.1% and vehicle and no treatment related changes.

The program did not include assessments of endothelial cell count. The draft guidance for industry for clinical developments of drugs, which has been issued after the design of this development program, requires such assessments (FDA draft guidance for Industry: Dry Eye: Developing Drugs for Treatment 2020).

This Phase 4 trial will be conducted to fulfill a post marketing requirement as determined by FDA to evaluate the corneal endothelial health of eyes treated with cyclosporine ophthalmic solution, 0.1%.

3 TRIAL OBJECTIVES

3.1 Primary objective

The primary objective of this trial is to evaluate the effect of cyclosporine ophthalmic solution, 0.1% on corneal ECD (cells/mm²) in comparison to a hypotonic saline solution in subjects with DED after 12 months of treatment.

3.2 Secondary objective

The secondary objective is to confirm long-term safety, endothelial health and tolerability of cyclosporine water-free ophthalmic solution, 0.1% in comparison to a hypotonic saline solution in subjects with DED.

4 TRIAL DESIGN

4.1 Overall Trial Design

This is a Phase 4 multi-center, randomized, double-masked, controlled clinical trial to evaluate the corneal endothelial health of DED subjects treated with cyclosporine ophthalmic solution, 0.1%. Approximately 200 subjects who meet all trial eligibility criteria will be randomized in a 2:1 ratio at approximately 6 sites to achieve at least 100 evaluable subjects treated with cyclosporine ophthalmic solution, 0.1% at 12 months.

Eligible subjects will dose the cyclosporine ophthalmic solution, 0.1% or saline solution, 0.6% bilaterally BID for approximately 12 months (52 weeks).

4.2 End of Trial Definition

The “end of the trial” for an individual subject is defined as that subject’s last visit as specified in the Schedule of Assessments.

The “end of the trial” for the overall trial is defined as completion of the last visit or procedure as specified in Schedule of Assessments for all subjects in the trial.

4.3 Visit Description

Subjects will be required to sign an Informed Consent before completing any trial related procedure. All examination procedures are listed in Section 8.4 and in the Schedule of Assessments. For consistency and to avoid interference, assessments should be performed in the

respective order as listed. Pregnancy testing can be performed at any time after informed consent and before randomization. IMP dispensation is described in Section 7.1.4.

Screening/Baseline Visit 1

All screening assessments must be performed before randomization, at baseline (Visit 1). Eligible subjects, who meet all eligibility criteria, will be randomized and given a supply of IMP until their next scheduled visit and a dosing diary to record that their doses were taken. [redacted]

Visits 2-5

Subjects will return to the clinic on week 12 ± 5 days (Visit 2), week 26 ± 5 days (Visit 3), week 40 ± 5 days (Visit 4), and week 52 ± 5 days (Visit 5) to assess trial parameters outlined in the Schedule of Assessments (Appendix 1). At those visits all used and unused trial medication should be returned to the clinic and a new trial medication kit will be dispensed at Visit 2, Visit 3, and Visit 4. The dosing diary will be collected during each visit and checked for compliance. Subjects will be discharged from the trial after all Visit 5 assessments have been completed.

Interim Telephone Calls

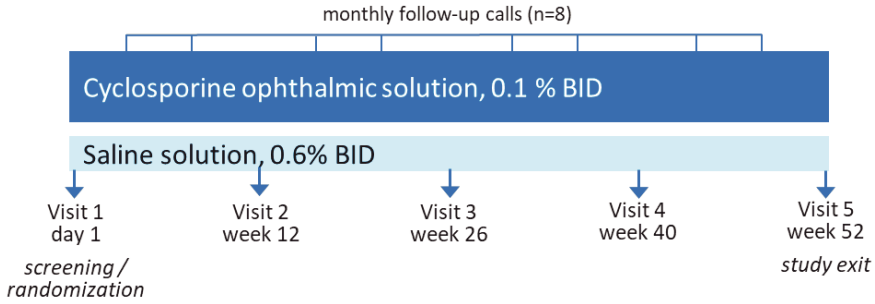
In order to keep a monthly contact with the subjects, interim telephone calls will be performed asking about the subject's well-being with non-leading AE questioning, BID dosing compliance, issues with IMP and any changes in medical history or concomitant treatments.

Early Termination (ET)

Subjects who terminate early during the treatment period will be asked to complete all assessments as indicated at Visit 5 on the Schedule of Assessments. In case an ocular surgery becomes necessary during the course of the trial, the subject shall be withdrawn, and an early term visit will be performed before the surgery. Dosing diary and trial medication will be collected. Subjects who are terminated early from the trial will not be replaced.

4.4 Trial Flow

Figure 2: Trial Flow



4.5 Enrollment and Treatment Assignment

Both treatment arms will be enrolled in parallel. Each subject will be assigned a unique subject number. All subject numbers will be assigned in sequential order by the site using a two (2) digit site number and three (3) digit subject number format (XX-XXX). If all inclusion and none of the exclusion criteria are met at Visit 1, each qualifying subject will be randomized at the same visit. Interactive Response Technology (IRT) will be used to assign the drug kit numbers at Visits 1-4. The subjects, Investigators, CRO personnel involved in conduct and monitoring of the trial and sponsor will be masked to IMP assignment.

4.6 Justification of Trial Design

The trial will be a randomized, double-masked, controlled trial to evaluate the corneal endothelial health of DED subjects treated with cyclosporine ophthalmic solution, 0.1%. The primary safety endpoint is the mean change from baseline in corneal ECD (count/mm^2) using specular microscopy at 12 months.

Randomization and double masking are state of the art measures to reduce bias. A control arm is considered necessary as there is a natural decrease in ECD over time. A hypotonic saline solution, 0.6% was selected as comparator, as the vehicle in case of the water-free cyclosporine ophthalmic solution, 0.1%, is a novel ingredient. A saline solution control allows for the ability to rule out potential effects on corneal endothelial cell health of both components of this novel cyclosporine ophthalmic solution, 0.1%, namely cyclosporine and perfluorobutylpentane.

Measuring ECD using specular microscopy is an accepted clinical safety endpoint in ophthalmology trials to assess non-invasively morphological and numerical corneal endothelial cell changes. Specular microscopy was the FDA required methodology to assess endothelial health in this study.

4.7 Justification for Dose

CYS-007 will use the same dose and schedule of application as recommended in the approved label of VEVYE.

5 TRIAL POPULATION

5.1 Number of Subjects (approximate)

Approximately [redacted] subjects will be screened to randomly assign 200 subjects in a 2:1 ratio to achieve at least 100 subjects treated with cyclosporine ophthalmic solution, 0.1% completing 52 weeks dosing with respective follow-up visits.

5.2 Trial Population Characteristics

All subjects must be at least 18 years of age, of either sex, and of any race. Furthermore, subjects must have a subject-reported history of DED in both eyes and meet all inclusion criteria and none of the exclusion criteria. As this is a safety study, all inclusion or exclusion criteria affecting the eye are always related to both eyes.

5.3 Inclusion Criteria

Subjects will be eligible to participate in this trial if they **meet all** following criteria:

1. Be at least 18 years of age;
2. Provide written informed consent;
3. Have a subject reported history of DED in both eyes [redacted]
4. Have an OSDI Score [redacted]
5. [redacted]
6. Have a baseline ECD [redacted]
7. Be able and willing to follow instructions, including participation in all trial assessments and visits.

5.4 Exclusion Criteria

Subjects will not be eligible to participate in this trial **if any** of the following criteria apply**:**
Subjects must not:

1. Have any clinically significant slit-lamp findings or ocular conditions at Visit 1 that require prescriptive topical 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;
2. [redacted]
3. Have a history of chronic or recurrent inflammatory eye disease (e.g., iritis, scleritis, uveitis, iridocyclitis, rubeosis iridis);

4. [redacted]
5. Have abnormal lid anatomy (e.g. incomplete eyelid closure, entropion, or ectropion) or abnormal blinking;
6. [redacted]
8. Have glaucoma or ocular hypertension (IOP > 20 mmHg) or have received a microinvasive glaucoma surgery (MIGS);
9. [redacted]
11. Be diagnosed with an ongoing ocular infection;
[redacted]
17. Have had intraocular surgery or ocular laser surgery within 365 days before Visit 1, or have any planned ocular surgeries during the trial period;
18. Have an uncontrolled systemic disease such as uncontrolled diabetes, severe renal insufficiency, progressive autoimmune diseases;
19. [redacted]
20. Be a woman who is pregnant, nursing, or planning a pregnancy;
21. Be a woman of childbearing potential who is not using an acceptable means of birth control; acceptable methods of contraception include: hormonal – oral, implantable, injectable, or transdermal contraceptives; mechanical – spermicide in conjunction with a barrier such as a diaphragm or condom; intrauterine device (IUD); or surgical sterilization of 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.
22. Be currently using an investigational drug or device or have used an investigational drug or device [redacted]

23. Have a condition or be in a situation (e.g. language barrier) 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.
24. Have previously participated in CYS-002, CYS-003, and/or CYS-004 CyclASol/VEVYE clinical trials
25. Live in the same household with another randomized CYS-007 participant.

[redacted]

5.6 Subject/ Trial Withdrawal Criteria

Subjects are free to discontinue their participation in the trial at any time without giving their reasons.

A subject **must be** discontinued from the trial for any of the following reasons:

- If at any time during the trial the Investigator determines that a subject's safety has been compromised;
- Occurrence of an exclusion criterion that is clinically relevant and affects the subject's safety;
- If discontinuation is considered necessary by the Investigator and/or sponsor;
- Occurrence of AEs that present an unacceptable consequence or risk to the subject in the judgment of the Investigator, sponsor, or the Medical Monitor;
- Occurrence of pregnancy;
- Withdrawal of subject`s consent.
- Necessity of an ocular surgery during the trial

If a subject has failed to attend scheduled trial assessments, the Investigator must determine the reasons and the circumstances as completely and accurately as possible.

In case a subject has to be withdrawn from the trial, the sponsor will be informed immediately. If there is a medical reason for withdrawal, the subject will remain under the supervision of the Investigator until satisfactory health has returned or the subject´s health has reached a stable condition.

Subjects who are withdrawn from the trial after dosing will not be replaced.

In case of premature withdrawal from the trial, the processes outlined in Section 8.4.2 should be followed. In any case, the appropriate electronic Case Report Form (eCRF) section including the reason for discontinuation as defined in Section 8.7.2 must be completed.

The trial **can be** prematurely discontinued as described in Section 8.8.

6 TRIAL PARAMETERS

6.1 Safety Endpoints

6.1.1 Primary Safety Endpoint

- Change from baseline (CFB) in corneal ECD (count/mm²) using specular microscopy at 12 months (Visit 5).

6.1.2 Secondary Safety Endpoints:

- ECD at month 3 and 12 and CFB at 3 months (Visit 2)
- Endothelial cell coefficient of variation [CV] (polymegethism) and CFB at each measured visit
- Endothelial cell hexagonality in % and CFB at each measured visit
- Central corneal thickness and CFB at each measured visit
- Ocular and non-ocular adverse events
- Visual acuity and CFB at each measured post-baseline visit
- Slit-lamp biomicroscopy at each measured post-baseline visit
- Intraocular pressure (IOP) and CFB at each measured post-baseline visit
- Dilated fundoscopy at each measured post-baseline visit

[redacted]

[redacted]

- [redacted]
- [redacted]
- [redacted]
- [redacted]

7 TRIAL MATERIALS

7.1 Investigational Medicinal Product

7.1.1 IMP/ Formulation

Table 1. Active Investigational Medicinal Product

	Investigational Product
Product name:	VEVYE (US brand name)
Chemical name:	Cyclosporine 0.1% [redacted]
Configuration:	[redacted]
Route of administration:	Topical ocular administration
Physical description:	Colorless and clear ophthalmic solution
Excipients:	[redacted]
Manufacturer:	[redacted]

Table 2. Control Investigational Product

	Control Investigational Product
Product name:	Saline solution
Chemical name:	Sodium chloride solution (0.6 %)
Molecular formula:	NaCl
Configuration:	[redacted]
Route of administration:	Topical ocular administration
Physical description:	Colorless and clear ophthalmic solution
Excipients:	[redacted]
Manufacturer:	[redacted]

7.1.2 Labeling and Packaging of IMP

IMP will be labelled according to the legal requirements and packaged into individual subject kits, each containing [redacted] bottles of cyclosporine ophthalmic solution, 0.1% or hypotonic saline solution, 0.6%. See Section 7.1.4 for details regarding dispensation to subjects. The subject should note the day of opening of individual bottles in the diary.

As per the Code of Federal Regulations 21 part 312, section 312.6, the labels for the IMP shall be comprised of:

- Protocol number
- Caution statement
- Kit or bottle number
- Storage conditions
- Name and address of the sponsor

7.1.3 IMP Storage

The IMP must be stored in a secure area accessible only to the Investigator or pharmacist and his/her designees. [redacted]

7.1.4 IMP Dispensation

[redacted] each site will be responsible for instructing subjects regarding IMP handling/administration, IMP dispensation and accountability, reviewing daily dosing diaries, and all discussions with study subjects related to IMP. Subjects will be instructed not to show or discuss the properties of the assigned Investigational Medicinal Product (IMP) and/or their experience with the IMP with other trial participants, and not to show or discuss the IMP with the Investigator or site staff [redacted], unless instructed to do so.

- The IMPs must only be distributed to subjects properly qualified under this protocol to receive IMP.
- [redacted]
- [redacted]
- [redacted]
- At Visits 2, 3, 4, and 5 used/unused IMP will be collected from subjects for IMP accountability [redacted]

Subjects will be instructed to immediately contact the site if there is any problem with the IMP, e.g. kit/bottle(s) was damaged or lost or if the open bottle was dropped. In case the subject needs a replacement bottle of IMP, the next bottle from the kit can be used by the subject. [redacted]

7.1.5 Instructions for Use and Administration

Subjects will be instructed on appropriate hygiene and eye drop dosing techniques for multiple use drops by the site's staff and given written instructions. They will then be instructed to instill one drop in each lower eyelid two times daily (in the morning and in the evening at bedtime) [redacted] Subjects will receive detailed written instruction on how to store and dose IMP, and how to complete their diary.

7.2 IMP Accountability

[redacted] each site must keep an accurate accounting of IMP received from the supplier by maintaining a detailed inventory. This includes the amount of IMP received by the site, amount dispensed to subjects, amount of IMP returned to the site by the subjects, and the amount returned to the sponsor or designee upon the completion of the trial.

Investigational trial medication orders, records of receipts including temperature monitoring data during shipment, dispensing records, and inventory forms will be examined and reconciled [redacted] At each visit, subjects will return all bottles [redacted] for accountability purposes. All IMP that is dispensed, returned to the site, and returned to the depot during the course of the trial must be accounted for on an IMP accountability form. At the conclusion of the study, accountability will be ascertained by performing reconciliation between the amount of IMP cartons (kits and their components/bottles) returned by the site and the depot receiving kits for destruction. No IMP or kits will be discarded prior to full accountability by sponsor's monitor.

7.3 IMP Handling and Disposal

Unless otherwise directed, at the end of the trial all returned used and unused IMP must be shipped from the clinical site to the depot for disposal of medications.
Note: The medications should not be disposed prior to full accountability by the sponsor's designated monitor.

The clinical site will provide a copy of all completed IMP accountability forms to the sponsor after the completion of the trial.

7.4 Other Trial Supplies

[redacted] Urine pregnancy test.

8 TRIAL METHODS AND PROCEDURES

8.1 Concurrent Medications and Therapies

Therapy considered necessary for the subject's welfare that will not interfere with the evaluation of the study medication may be given at the discretion of the Investigator. If there is any question as to whether the medication may interfere, the Investigator should contact the Medical Monitor or Sponsor.

[redacted]

8.2 Prohibited Treatments

Disallowed treatments are listed in the Exclusion Criteria (Section 5.4). [redacted]

8.3 Restrictions and Prohibitions

If a visual disturbance occurs upon drop instillation, the subject should be advised not to drive or use machines until such effects have disappeared.

8.4 Examination Procedures

8.4.1 Procedures to be Performed at Each Trial Visit with Regard to Trial Objective(s)

The procedures outlined in this section will be performed as described in Appendix 1: Schedule of Assessments

and Appendix 2: Examination Procedures, Tests, Equipment, and Techniques

Visit 1 (Day 1):

- Informed consent/HIPAA
- Medical/medication history update
- Review of qualification criteria (in-/exclusion criteria)
- Urine pregnancy test for females of childbearing potential
- Specular microscopy
- [redacted]
- Visual acuity
- Slit-lamp biomicroscopy
- [redacted]
- Intraocular pressure
- Dilated fundoscopy
- Non-leading AE questioning
- Randomization
- Dispensation of IMP and dosing diary
- IP administration in clinic
- Schedule for Visit 2

Visit 2 (Week 12 ± 5 days)

- At the beginning of the visit the subject will be asked about the time of last dose.
  [redacted]
- Collection and review of IMP and dosing diary
  - [redacted]
  - [redacted]
- Medical/medication history update
- Specular microscopy
- [redacted]
- Visual acuity

- Slit-lamp biomicroscopy
- [redacted]
- Non-leading AE questioning
- Dispensation of IMP and dosing diary
- Schedule for Visit 3

Visit 3 (week 26 ± 5 days) and Visit 4 (week 40 ± 5 days)

- At the beginning of the visit the subject will be asked about the time of last dose. [redacted]
- Collection and review of IMP and dosing diary;
  - [redacted]
  - [redacted]
- Medical/medication history update
- [redacted]
- [redacted]
- Visual acuity
- Slit-lamp biomicroscopy
- Non-leading AE questioning
- Dispensation of IMP and dosing diary
- Subjects are scheduled for next visit

Visit 5 (week 52 ± 5 days) or ET

- At the beginning of the visit the subject will be asked about the time of last dose. [redacted]
- Collection and review of IMP and dosing diary;
  - [redacted]
  - [redacted]
- Medical/Medication history update
- Urine pregnancy test for females of childbearing potential
- Specular microscopy
- [redacted]
- [redacted]
- Visual acuity
- Slit-lamp biomicroscopy
- [redacted]
- Intraocular pressure
- Dilated fundoscopy
- [redacted]
- Non-leading AE questioning
- Study Exit

TC 1-8 (Weeks 4/ 8/ 16/ 21/ 30/ 36/ 44/ 48 ± 7 days)

- Medical /Medication History Update
- Non-leading AE questioning
- Confirm dosing as instructed

8.4.2 Early Termination/Discontinuations

Data from subjects discontinuing after dosing will be captured completely in the eCRF including Early Termination Visit and reason for discontinuation.

If a dosed subject is discontinued from the trial before Visit 5 and is not willing to perform all subsequent visits and assessments, all evaluations of Visit 5 (at least all safety related evaluations) should be performed at the Early Termination Visit on the day of early termination/ discontinuation or at the discretion of the Investigator.

In case ocular surgery becomes necessary during the trial the subject is to be withdrawn from the trial and an ET visit shall be performed before the surgery.

8.4.3 Unscheduled Visits

An Unscheduled Visit may be performed during the trial to ensure subject safety. All procedures performed at an Unscheduled Visit will be recorded in the source documents and on the Unscheduled Visit eCRF pages. Any Unscheduled Visit procedure listed in the eCRF that is not performed should be indicated as “not done”.

Evaluations that may be conducted at an Unscheduled Visit include:

- Slit-lamp biomicroscopy
- Specular microscopy
- Visual acuity
- Intraocular pressure
- Urine pregnancy test (for women of childbearing potential)
- Dilated fundoscopy
- Assessment of AEs
- Assessment of concomitant medications and/or treatments
- Any other assessments needed in the judgement of the Investigator
- IMP dispensation (as needed)

8.5 Endothelial Cell Density Assessment and Analysis

All clinical sites need to be equipped with the same model of a specular microscope [redacted] to collect corneal endothelial images [redacted]. Specular microscopes will be calibrated prior to shipment to site and study data collection. Delegated personnel at the investigational site will be trained for collection of high-quality images.

Specular microscopy imaging should be performed according to the training and respective manual provided [redacted]. [redacted]

8.6 Compliance with Dosing/Protocol Deviations

Subjects will be instructed on the proper use and storage of the IMP at Visits 1, 2, 3, and 4 and provided with written instructions. Subject diaries and IMP will be collected at each visit from Visit 2 up to and including Visit 5 to assess subject compliance with the protocol.

[redacted]

A protocol deviation is any noncompliance with the clinical study protocol, GCP, or Study Operations manual requirements. Noncompliance may be on the part of the subject, the PI, or study staff. As a result of deviations, corrective actions may need to be developed by the study staff and implemented promptly.
These practices are consistent with PI and Sponsor obligations in ICH GCP guidelines:

- Compliance with Protocol, Sections 4.5.1, 4.5.2, 4.5.3 and 4.5.4
- Quality Assurance and Quality Control, Section 5.1.1
- Noncompliance, Sections 5.20.1 and 5.20.2

All deviations from the protocol must be addressed in study subject source documents and promptly reported to the IRB according to their requirements.

8.7 Subject Disposition

8.7.1 Completed Subjects

A completed subject is one who has not been discontinued from the trial and who has completed the final study visit (Visit 5).

8.7.2 Discontinued Subjects

Notification of a subject's discontinuation and the reason for discontinuation will be made to *CRO* and/or trial sponsor and will be clearly documented on the eCRF as:

- Adverse Events
- Protocol violations
- Lack of efficacy
- Administrative reasons (e.g., inability to continue, lost to follow up)
- Sponsor termination of trial
- Subject choice (e.g. withdrawal of consent)
- Other

Subjects must be discontinued as outlined in Section 5.6.

Subjects, who discontinue for any reason after dosing will not be replaced.

8.8 Trial Termination

The whole trial may be discontinued prematurely in the event of any of the following:

- New information leading to unfavorable risk-benefit judgment of the IMP, e.g., due to:
  - Occurrence of significant previously unknown adverse reactions or unexpectedly high intensity or incidence of known adverse reactions
    or
  - Other unfavorable safety findings.
- Sponsor's decision that continuation of the trial is unjustifiable for medical or ethical reasons.
- Terminated or suspended upon request of Health Authorities

Health Authorities and Institutional Review Boards (IRBs)/ Independent Ethics Committees (IECs) will be informed about the discontinuation of the trial in accordance with applicable regulations.

8.9 Trial Duration

An individual subject's participation will involve 5 visits and 8 phone calls over approximately a 52-week period. After the trial, subjects will be treated according to the standard of care at the discretion of the treating physician. The total duration of the trial from "first subject in" to "last subject out" is expected to be 18 months.

9 ADVERSE EVENTS

9.1 Adverse Event

An adverse event (AE) is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not the event is considered IMP-related. An AE can therefore be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease occurring after the subject signed informed consent.

If there is a worsening of a medical condition that was present prior to the administration of the IMP, this should also be considered a new AE and reported. Any medical condition present prior to the administration of the IMP that remains unchanged or improved should not be recorded as an AE at subsequent visits.

Worsening of DED will be considered an AE only if the DED status of the subject exceeds their previous experiences with the condition. This will be determined by the subject and the Investigator.

IMP includes the investigational drug under evaluation, the control or any other medications required by the protocol given during any stage of the trial.

Documentation regarding the AE should be made as to the nature, date of onset, end date, severity, relationship to IMP, expectedness, action(s) taken, seriousness, and outcome of any sign or symptom observed by the physician or reported by the subject upon non-leading questioning.

AEs (both elicited and observed) will be monitored from the time of Informed Consent and throughout the trial. All AEs will be promptly reviewed by the Investigator for accuracy and completeness. All AEs will be documented on the appropriate source document and eCRF.

9.1.1 Severity

Severity of an AE is defined as a qualitative assessment of the degree of intensity of an AE as determined by the Investigator or reported to him/her by the subject. The assessment of severity is made irrespective of relationship to investigational product or seriousness of the event and should be evaluated according to the following scale:

- *Mild*: Event is noticeable to the subject but is easily tolerated and does not interfere with the subject's daily activities.
- *Moderate*: Event is bothersome, possibly requiring additional therapy, and may interfere with the subject's daily activities.
- *Severe*: Event is intolerable, necessitates additional therapy or alteration of therapy, and interferes with the subject's daily activities.

Severity is not the same as "seriousness", which is based on the outcome or action criteria usually associated with events that pose a threat to life or functioning (see Section 9.2). Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

9.1.2 Relationship to Investigational Product

The relationship of each AE to the IMP should be determined by the Investigator using these explanations:

- *Suspected*: A reasonable possibility exists that the investigational product caused the AE.
- *Not Suspected*: A reasonable possibility does not exist that the investigational product caused the AE.

Suspected adverse reaction means any AE for which there is a reasonable possibility that the investigational product caused the AE. "Reasonable possibility" means there is evidence to suggest a causal relationship between the IMP and the AE. Types of evidence that would suggest a causal relationship between the IMP and the AE include: a single occurrence of an event that is uncommon and known to be strongly associated with IMP exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome); one or more occurrences of an event that is not commonly associated with IMP exposure, but is otherwise uncommon in the population exposed to the IMP (e.g., tendon rupture); an aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the trial population independent of drug therapy) that indicates those events occur more frequently in the IMP-treatment group than in a concurrent or historical control group.

9.1.3 Expectedness

The expectedness of an AE should be determined based upon existing safety information about the IMP. The IMP contains the active ingredient cyclosporine and the vehicle F4H5 and has been tested in five clinical studies under Novaliq's sponsorship up to now.

In lieu of an Investigator's Brochure, the Investigator is referred to the VEVYE prescribing information for risk and benefit details. The most frequent AEs have been listed in the prescribing information of the approved product VEVYE. Therefore, the following definition will be used:

- *Unexpected*: An AE that is not listed in the VEVYE prescribing information in the Adverse Reaction Section.
- *Expected*: An AE that is listed in the VEVYE prescribing information in the Adverse Reaction Section at the specificity and severity that has been observed.
- *Not Applicable*: Any AE that is unrelated to the IMP.

The Investigator should initially classify the expectedness of an AE, but the final classification is subject to the sponsor's determination.

9.1.4 Outcome

The outcome of any AE will be determined and recorded using the following categories:

- Recovered/Resolved
- Recovering/Resolving
- Not Recovered/Not Resolved
- Recovered/Resolved with Sequelae
- Fatal
- Unknown

9.1.5 Period of Observation

For the purpose of this study, the period of observation for collection of adverse events extends from the time the subject gives informed consent until the end of Visit 5 (Week 52 ± 5 days) or the Early Termination Visit.

If the Investigator detects a Serious Adverse Event (SAE) (Section 9.2) in a study subject after the end of the period of observation this should be reported to the Sponsor only if the Investigator considers the event related to prior study treatment or procedures. The Investigator should contact the sponsor to determine how the adverse event should be documented and reported.

9.2 Serious Adverse Events

An AE is considered serious if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death;
- A life-threatening AE;
    - Note: An AE is considered "life-threatening" if, in the view of either the Investigator or sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization;
    - Note: The term "inpatient hospitalization" refers to any inpatient admission (even if less than 24 hours). For chronic or long-term inpatients, inpatient admission includes transfer within the hospital to an acute/intensive care inpatient unit. Inpatient hospitalization does not include: emergency room visits; outpatient/same-day/ambulatory procedures; observation/short stay units; rehabilitation facilities; hospice facilities; nursing homes; or clinical research/phase 1 units.
    - Note: The term "prolongation of existing hospitalization" refers to any extension of an inpatient hospitalization beyond the stay anticipated or required for the reason for the initial admission as determined by the Investigator or treating physician.
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
    - Note: A SAE specifically related to visual threat would be interpreted as any potential impairment or damage to the subject's eyes (e.g., hemorrhage, retinal detachment, central corneal ulcer or damage to the optic nerve).

- A congenital anomaly/birth defect;
- Medically important.
    - Note: Important medical events that may not result in death, are life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

9.3 Procedures for Reporting Adverse Events

All SAEs and their outcomes must be reported to CRO, Novaliq, [redacted] and the IRB as required by the IRB, federal, state, or local regulations and governing health authorities and recorded on the appropriate eCRF.

9.3.1 Reporting a Serious Adverse Event

To ensure subject safety, all SAEs, regardless of their relationship to the IMP, must be immediately reported (i.e., within a maximum 24 HOURS after becoming aware of the event). All information relevant to the SAE must be recorded on the appropriate SAE report forms. The Investigator is obligated to pursue and obtain information requested by [redacted] CRO and/or Novaliq in addition to that information reported on the case report form. All subjects experiencing a SAE must be followed up and the outcome reported.

In the event of a SAE, the Investigator must notify [redacted] the sponsor immediately; obtain and maintain in his/her files all pertinent medical records, information, and medical judgments from colleagues who assisted in the treatment and follow-up of the subject; provide [redacted] CRO and the trial sponsor with a complete case history, which includes a statement as to whether the event was or was not suspected to be related to the use of the IMP; and inform the IRB of the AE within their guidelines for reporting SAEs.

Contact information for reporting SAEs:

[redacted]

Contact information for Medical Monitor:

[redacted]

9.3.2 Reporting a Suspected Unexpected Serious Adverse Reaction

All SAE that are ‘suspected reactions’ and ‘unexpected’ are to be reported to the IRB as required by the IRB, federal, state, or local regulations and governing health authorities.

9.4 Procedures for Unmasking of IMP

All subjects, Investigators, and trial personnel involved with the conduct of the trial will be masked with regard to treatment assignments. The mask may be broken in exceptional circumstances.

In a medical emergency, when the management of a subject's condition requires knowledge of the treatment assignment, the Investigator, or designee, will obtain the trial treatment assignment from the IRT. If possible, the medical emergency should be discussed with the Medical Monitor prior to obtaining the treatment assignment, or as soon after as possible. The sponsor may unmask for regulatory submission determination of an SAE when necessary (see Section 9.3.1).

In a non-emergency situation, when a code break is required, it must be discussed with the Medical Monitor and the trial sponsor. The code break must be approved in writing by the sponsor.
If the randomization code for a subject is broken, the Investigator will record the date and reason for lifting the mask for that subject in the source documents. Upon unmasking, the subject will be withdrawn from the trial and should complete the Early Termination procedures.

9.5 Type and Duration of the Follow-up of Subjects after Adverse Events

The Investigator will follow unresolved AEs to resolution until the subject is lost to follow-up or until the AE is otherwise classified. Resolution means the subject has returned to baseline state of health or the Investigator does not expect any further improvement or worsening of the AE. If the subject is lost to follow-up, the Investigator should make 3 reasonable attempts to contact the subject via telephone, post, or certified mail. All follow-up will be documented in the subject's source document. Non-serious AEs identified on the last scheduled contact must be recorded on the AE eCRF page with the status noted. If the Investigator becomes aware of any new information regarding an existing SAE (i.e., resolution, change in condition, or new treatment), a new SAE Report Form must be completed and e-mailed to [redacted] within 24 hours of the site's awareness of the new information. The original SAE form is not to be altered. The report should describe whether the event has resolved or continues and how the event was treated.

9.6 Procedures for Reporting Pregnancies

Pregnancy in itself is not considered an AE or SAE (unless there is a suspicion that an IMP may have interfered with the effectiveness of a contraceptive medication), but it is an important medical event that must be followed up. Any pregnancy that occurs during the clinical trial where the fetus could have been exposed to IMP must be followed through the outcome of the pregnancy.

It is the responsibility of the Investigator to obtain the outcome and condition of the infant information within 30 calendar days after the initial notification and approximately 30 calendar days postpartum.

If a subject or Investigator suspects that the subject may be pregnant prior to IMP administration, the IMP must be withheld until the results of pregnancy testing are available. If pregnancy is confirmed, the subject must not receive IMP and must not be enrolled in the study. If pregnancy is suspected while the subject is receiving IMP treatment, the IMP must immediately be withheld until the result of pregnancy testing is known.

If a female has a positive pregnancy test during the trial, then the Investigator must report the pregnancy and the outcome of the pregnancy to [redacted] within 24 hours of learning about the pregnancy.

A Pregnancy Reporting Form will be completed by the trial site's Principal Investigator and sent to [redacted] via the SAE e-mail (see Section 9.3). [redacted] will forward the documentation to the Medical Monitor, CRO project manager and the sponsor for review. An Early Termination Visit should be performed (see Section 5.6).

At the completion of the pregnancy, the Pregnancy Outcome Form is to be submitted to CRO via the SAE contact details. [redacted] will manage the query and reconciliation process until the pregnancy documentation is complete.

10 STATISTICAL HYPOTHESES AND METHODS OF ANALYSES

10.1 Analysis Populations

Safety Population: The safety population includes all subjects who have received at least one dose of the investigational product. The safety population will be analyzed for all assessments. Subjects in the safety population will be analyzed as treated.

The statistical analysis of ECD and other endothelial parameters will be performed on the safety population using all observed data.

10.2 Statistical Hypotheses

This study is designed to obtain and evaluate corneal ECD in at least 100 evaluable participants per regulatory request. Therefore, there are no pre-stated statistical hypotheses.

10.3 Sample Size

Assuming a dropout rate [redacted] in this one-year trial, approximately 200 subjects will be enrolled with a 2:1 randomization ratio (cyclosporine ophthalmic solution, 0.1%: saline solution, 0.6%) to ensure that at least 100 evaluable participants treated with cyclosporine ophthalmic solution, 0.1% complete the 52 weeks (12-month) treatment period.

10.4 Statistical Analysis

10.4.1 General Considerations

Quantitative variables will be summarized using number of subjects (n), mean, median, standard deviation, minimum and maximum. The dichotomous variables will be summarized using counts and percentages.

All summaries will be presented by treatment groups and provided for demographics, baseline medical history, concurrent therapies, and subject disposition.

For the purpose of summarization, medical history, concurrent therapies, and AEs will be coded to MedDRA and WHO Drug dictionaries, as appropriate.

Baseline measures are defined as the measure on the baseline visit. CFB will be calculated as Visit – Baseline. All primary and secondary analyses will be two-sided at a significance level of 0.05.

10.4.2 Unit of Analysis

The primary analysis is done on the “study eye” which is defined as the right eye (OD). The fellow eye is the left eye (OS). All secondary analysis will be done for both eyes.

10.4.3 Missing Data

The primary analysis will be completed with available data per subject from the Safety Set (SAF).

10.4.4 Primary Safety Analyses

ECD (cells/mm^2) and CFB in the study eye will be summarized by treatment group and follow-up visit using the sample size, mean, standard deviation, median, minimum, and maximum.

[redacted]

10.4.5 Secondary Safety Analyses

All safety endpoints captured using specular microscopy will be analyzed and summarized using the same descriptive methodology as the primary analysis. All other visit-based safety data will be analyzed by visit and treatment using change or shift from baseline, where applicable. Assessments performed by eye (study eye and fellow eye) will be summarized separately. Shifts from baseline to on-treatment value for ocular safety assessments will be summarized. Secondary analyses will be based on observed data only.

Other safety endpoints including visual acuity, slit-lamp biomicroscopy, intraocular pressure and dilated fundoscopy will be summarized by visit and treatment using descriptive statistics. Changes or shifts from baseline will also be summarized where appropriate. Assessments performed by eye will be summarized separately for study eye and fellow eye.

Dosing information will be summarized for all subjects by treatment and listed for each subject. Discontinuation of treatment will be summarized by treatment received. The primary reason for trial drug discontinuation will also be summarized by treatment received.

AEs will be coded using the MedDRA dictionary. An AE is treatment emergent if it occurs or worsens after the first dose of trial treatment.

Frequencies and percentages of subjects with treatment-emergent adverse events (TEAEs), serious TEAEs, and TEAEs causing premature discontinuation will be provided. Furthermore, frequencies will be given of subjects with TEAEs by system organ class, by system organ class and preferred term, by system organ class, preferred term and maximal severity, by system organ class, preferred term and strongest relationship, and by system organ class, preferred term, maximal severity, and strongest relationship. Separate summaries will be performed for ocular and non-ocular AEs.

Concomitant medications will be coded using the most recent version of WHO-Drug Dictionary and summarized by treatment group.

[redacted]

11 COMPLIANCE WITH GOOD CLINICAL PRACTICES, ETHICAL CONSIDERATIONS, AND ADMINISTRATIVE ISSUES

This trial will be conducted in compliance with the protocol, current Good Clinical Practices (GCPs), including the International Conference on Harmonization (ICH) Guidelines, and in general, consistent with the Declaration of Helsinki. In addition, all applicable local, state, and federal requirements relevant to the use of investigational products in the countries involved will be adhered to.

11.1 Protection of Human Subjects

11.1.1 Subject Informed Consent

Informed consent/assent must take place before any trial specific procedures are initiated. Signed and dated written informed consent must be obtained from each subject prior to performing any trial specific procedures and assessments.

All informed consent/assent forms must be approved for use by the sponsor and receive approval/favorable opinion from an IRB prior to their use. If the consent form requires revision (e.g., due to a protocol amendment or significant new safety information), it is the Investigator's responsibility to ensure that the amended informed consent is reviewed and approved by the governing IRB and that it is read, signed, and dated by all subjects subsequently enrolled in the trial as well as those currently enrolled in the trial.

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11.1.2 Institutional Review Board (IRB) Approval

This trial is to be conducted in accordance with Institutional Review Board regulations (U.S. 21 CFR Part 56.103).

Only an IRB/IEC approved version of the informed consent form will be used.

11.2 Ethical Conduct of the Trial

This trial will be conducted in accordance with the ethical principles that originated with the Declaration of Helsinki.

11.3 Subject Confidentiality

All personal trial subject data collected and processed for the purposes of this trial should be maintained by the Investigator and his/her staff with adequate precautions as to ensure that the confidentiality of the data in accordance with local, state, and federal laws and regulations.

Monitors, auditors, and other authorized representatives of CRO, the external safety provider, the sponsor, the IRB/IEC approving this trial, the FDA, the Department of Health and Human Services, other domestic government agencies, and other foreign regulatory agencies will be granted direct access to the trial subject's original medical and trial records for verification of the data and/or clinical trial procedures. Access to this information will be permitted to the aforementioned individuals to the extent permitted by law.

A report of the results of this trial may be published or sent to the appropriate health authorities in any country in which the investigational product may ultimately be marketed, but the subject's identity will not be disclosed in these documents.

11.4 Documentation

Source documents may include a subject's medical records, hospital charts, clinic charts, the Investigator's trial subject files, as well as the results of diagnostic tests. The Investigator's copy of the eCRFs serves as the Investigator's record of a subject's trial-related data.

11.4.1 Retention of Documentation

All trial related correspondence, subject records, consent forms, record of the distribution and use of all investigational products and copies of case report forms should be maintained on file for at least two years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region; or until at least two years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents will be retained for a longer period if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the Investigator/institution as to when these documents no longer need to be retained. The Investigator must notify the sponsor prior to destroying trial documentation even after the above-mentioned time has passed.

If the responsible Investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping trial records, custody must be transferred to a person who will accept the responsibility. The sponsor must be notified in writing of the name and address of the new custodian.

11.5 Recording of Data on Source Documents and Electronic Case Reports Forms (eCRFs)

All subject data will be captured in the subject paper or electronic source which will be transcribed/transferred to the eCRFs. The Investigator is responsible for ensuring that trial data

is completely and accurately recorded on each subject's eCRF, source document, and all trial-related materials. All trial data should also be attributable, legible, contemporaneous, and original. Recorded data should only be corrected in a manner that does not obliterate, destroy, or render illegible the previous entry (e.g., by drawing a single line through the incorrect entry and writing the revision next to the corrected data). An individual who has corrected a data entry should make clear who made the correction and when, by adding to the correction his/her initials as well as the date of the correction.

Data entry of all enrolled subjects will use software that conforms to 21 CFR Part 11 requirements and will be performed only by staff that have been trained on the system and have access to the system. An audit trail will be maintained within the electronic system to capture all changes made within the eCRF database. After the end of the trial and database lock, applicable subjects' eCRFs will be provided in a 21 CFR Part 11 compliant way to each Investigator site to be maintained on file by the Investigator.

11.6 Monitoring and Quality Assurance

During the course of the trial a clinical research associate (CRA) will review protocol compliance, assess IMP accountability or reviews data to ensure quality, integrity and the trial is being conducted according to the pertinent regulatory requirements. The review of the subjects' medical records will be performed in a manner that adequately maintains subject confidentiality. Further details of the trial monitoring will be outlined in a monitoring plan.

Domestic and foreign regulatory authorities, CRO and quality assurance, sponsor and/or its designees may carry out on-site inspections and/or audits which may include source data checks. Therefore, direct access to the original source data will be required for inspections and/or audits. All inspections and audits will be carried out with consideration to data protection as well as subject confidentiality to the extent that local, state, and federal laws apply.

11.7 Handling of Biological Samples

Not applicable.

11.8 Publications

Authorship and manuscript composition will reflect cooperation among all parties involved in the trial. Authorship will be established before writing the manuscript. The trial sponsor will have the final decision regarding authorship, manuscript, and publication.

12 REFERENCES

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