# Clinical Trial Protocol: CYS-004

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| **Protocol Title:** | A Phase 3, multi-center, randomized, double-masked, vehicle-controlled clinical trial to assess the efficacy and safety of topical CyclASol® for the treatment of Dry Eye Disease |
| **Protocol Number:** | CYS-004 / ESSENCE 2 |
| **Trial Phase:** | 3 |
| **Investigational Product Name:** | CyclASol® 0.1 % Ophthalmic Solution (Cyclosporine A 0.1%) |
| **IND Number:** | 128163 |
| **Indication:** | Dry Eye Disease (*keratoconjunctivitis sicca*) |
| **Sponsor:** | Novaliq GmbH<br>Im Neuenheimer Feld 515<br>69120 Heidelberg<br>Germany |
| **Contract Research Organization:** | [redacted] |
| **Original Protocol:** | 27th August 2020 |
| **Amendment 1:** | 2nd December 2020 |
| **Amendment 2:** | 15th March 2021 |

**Confidentiality Statement**

This protocol contains confidential, proprietary information of [redacted] 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**

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| **Scientific Lead:** | [redacted] |
| **Clinical Operations:** | [redacted] |

**MEDICAL MONITOR**

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| **Clinical Project Manager:** | [redacted] |
| **Biostatistician:** | [redacted] |

## 1.2 SYNOPSIS

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| **Protocol Title:** | A Phase 3, multi-center, randomized, double-masked, vehicle-controlled clinical trial to assess the efficacy and safety of topical CyclASol® for the treatment of Dry Eye Disease |
| **Protocol Number:** | CYS-004 / ESSENCE 2 |
| **Investigational Medicinal Product:** | 1) CyclASol 0.1% Ophthalmic Solution (Cyclosporine A 0.1% solution)<br>2) Vehicle Ophthalmic Solution (F4H5) |
| **Trial Phase:** | 3 |
| **Trial Objective** | The objective of this trial is to assess the efficacy, safety, and tolerability of CyclASol 0.1% Ophthalmic Solution in comparison to the vehicle for the treatment of the signs and symptoms of Dry Eye Disease (DED). |
| **Overall Trial Design:** | |
| **Structure:** | This clinical trial is multi-center, randomized, double-masked, and vehicle-controlled with a run-in period with artificial tears. |
| **Participant Duration:** | An individual subject's participation is estimated to be approximately 6 weeks (approx. 2 weeks screening and 4 weeks treatment period). |
| **Trial Duration** | The estimated trial duration is 8 months, from first subject first visit to last subject last visit. |
| **Controls:** | Vehicle (F4H5) |
| **Dosage/Dose Regimen:** | Eligible subjects will be randomized to one of the following treatments to be administered BID bilaterally for approximately 29 days (from Visit 1 to Visit 3).<br>• CyclASol 0.1% Ophthalmic Solution (Cyclosporine A 0.1% solution)<br>• Vehicle (F4H5)<br>A 14-day run-in period will be used for subject selection before randomization. During this period, all subjects will receive Systane® Balance to administer bilaterally BID. |
| **Summary of Visit Schedule:** | 4 visits over the course of approximately 6 weeks:<br>• Visit 0, Day -14 ± 2 days, Screening;<br>• Visit 1, Day 1, Baseline/Randomization;<br>• Visit 2, Day 15 ± 2 days, 2-Week Follow-Up;<br>• Visit 3, Day 29 ± 2 days, 4-Week Follow-Up and Trial Exit |
| **Measures Taken to Reduce Bias:** | This is a multi-center, randomized, double-masked and vehicle-controlled clinical trial. |
| **Trial Population Characteristics:** | |
| **Number of Subjects:** | Approximately 1800 subjects will be screened to enroll at least 834 (417 per treatment arm) subjects at approximately 25 sites. |
| **Condition/Disease:** | Dry Eye Disease (*keratoconjunctivitis sicca*) |
| **Inclusion Criteria:** | **Each subject must:**<br>1. Be at least 18 years of age; |

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| | 2. Provide written informed consent;<br>3. Have a subject reported history of Dry Eye Disease in both eyes for at least 180 days before Visit 0;<br>4. 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;<br>5. Have a Dryness Score ≥ 50 (VAS) at Visit 0 and Visit 1;<br>6. Have a total corneal fluorescein staining (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;<br>7. 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;<br>8. Have an unanesthetized Schirmer's Test score between 1 mm and 10 mm inclusive at Visit 0 and Visit 1;<br>9. Have at least one eye, the same eye, satisfy inclusion criteria 6, 7, and 8; and<br>10. Be able and willing to follow instructions and participate in all trial assessments and visits. |
| **Exclusion Criteria:** | **Each subject must not:**<br>1. 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;<br>2. 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;<br>3. Had Lipiflow procedures performed during the past 180 days prior to Visit 0;<br>4. Have abnormal lid anatomy (e.g. incomplete eyelid closure, entropion, or ectropion) or abnormal blinking;<br>5. 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);<br>6. Have an ocular or periocular malignancy; |

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| | 7. Have a corneal epithelial defect, or have in more than 2 of the 5 corneal regions > 50% confluent corneal staining;<br>8. Have a history of herpetic keratitis;<br>9. Have active ocular allergies or ocular allergies that may become active during the trial period;<br>10. Have worn contact lenses within 90 days before Visit 0 or anticipate using contact lenses during the trial;<br>11. 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;<br>12. Be diagnosed with an ongoing ocular infection;<br>13. 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;<br>14. Have active ocular or periocular rosacea or a pterygium;<br>15. 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;<br>16. Have used topical CsA or Lifitegrast within 60 days before Visit 0;<br>17. Have used any topical antiglaucoma medications (including prostaglandin analogues applied to eyelid margin) within 90 days before Visit 0;<br>18. 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;<br>19. Have used any topical ocular or facial steroids, or serum tears or oral doxycycline, or oral tetracycline within 30 days before Visit 0;<br>20. 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;<br>21. 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;<br>22. 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; |

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| | 23. Have been randomized in a previous CyclASol trial;<br>24. Be a woman who is pregnant, nursing, or planning a pregnancy;<br>25. 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);<br>26. 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;<br>27. Have an uncontrolled systemic disease;<br>28. Have a known allergy or sensitivity to the IMP or its components: Cyclosporine A (CsA) or semifluorinated alkanes (SFA);<br>29. 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<br>30. 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. |
| **Trial Formulations and Formulation Numbers:** | • Systane Balance (run-in)<br>• CyclASol 0.1% Ophthalmic Solution (Cyclosporine A 0.1% solution)<br>• Vehicle (F4H5) |
| **Evaluation Criteria:** | |
| **Efficacy Endpoints:** | **Primary Efficacy Endpoints:**<br>1. Change from baseline (CFB) in tCFS score (NEI scale) at Day 29<br>2. CFB in Dryness Score (VAS) at Day 29 |

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| | **Key Secondary Efficacy Endpoints:**<br>• CFB in total conjunctival lissamine green staining score (Oxford scale) at Day 29<br>• Proportion of responders in central corneal fluorescein staining (cCFS) score (≥ 1 score improvement on NEI scale) at Day 29<br>• Proportion of responders in tCFS score (≥ 3 score improvement on NEI scale) at Day 29<br>• CFB in cCFS score (NEI scale) at Day 29<br>• CFB in tCFS (NEI scale) at Day 15<br>• CFB in visual analogue scale (VAS) for blurred vision at Day 29<br><br>**Secondary Efficacy Endpoints:**<br>• cCFS score and CFB at Day 15<br>• 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)<br>• Dryness Score and blurred vision VAS at each post-baseline visit and CFB to Day 15<br><br>**Other Prespecified Efficacy Endpoints:**<br>• Reading assessments (MNRead; sustained silent reading test; IReST) and CFB at each post-baseline visit<br>• CFS score per sub-regions (other than cCFS; NEI scale) and CFB to each measured post-baseline visit<br>• Proportion of responders in tCFS score (≥ 2 and ≥ 4 scores improvement on NEI scale) at Day 15 and Day 29<br>• VAS for frequency of dryness, awareness of dryness, reading problems, fluctuating vision, looking at screens and driving at night and CFB at each measured post-baseline visit<br>• Ocular Surface Disease Index (OSDI) total, individual and subtotal scores and CFB at each measured post-baseline visit<br>• Unanesthetized Schirmer's Test and CFB at each measured post-baseline visit<br>• Proportion of responders in unanesthetized Schirmer's Test (≥ 10 mm increase) at each measured post-baseline visit<br>• Tear Film Break-Up Time (TFBUT) and CFB at each measured post-baseline visit |

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| **Safety Endpoints:** | • Visual acuity and CFB at each measured post-baseline visits;<br>• Slit-lamp biomicroscopy findings and CFB at each measured post-baseline visits<br>• Intraocular pressure and CFB at each measured post-baseline visits<br>• Dilated fundoscopy findings and CFB at each measured post-baseline visits<br>• Systemic and ocular AEs |
| **Other:** | • Drop Comfort Scale and Questionnaire<br>• Eyedrop Acceptability Questionnaire |

**General Statistical Methods and Types of Analyses**

Sample Size:

The trial has been designed to have 90% power assuming independence between the sign and symptom primary efficacy endpoints; positive correlation between these two endpoints would increase the overall power. Accounting for subject discontinuations, approximately 834 total subjects (417 per treatment arm) will be enrolled assuming a dropout rate of approximately 10%.

Multiplicity Considerations:

Hierarchical fixed sequence testing will be employed to maintain the type I error rate. The primary analyses will first test the difference in the mean CFB corneal fluorescein staining (NEI scale) total score between treatments at Day 29. If the test of the difference is statistically significant at the two-sided alpha = 0.05 level in favor of CyclASol , then the trial will be considered a success; CyclASol will be declared to be superior to vehicle in the mean CFB corneal fluorescein staining (NEI scale) total score at Day 29; and the difference in the mean CFB Dryness Score (VAS) between treatments at Day 29 will be tested at the two-sided alpha = 0.05 level.

If in addition to a statistically significant test of the difference in the mean CFB tCFS score (NEI scale) at Day 29 in favor of CyclASol, the test of the difference in the mean CFB Dryness Score (VAS) at Day 29 is also statistically significant in favor of CyclASol, then CyclASol will be declared to be superior to vehicle in both the mean CFB tCFS score (NEI scale) and the mean CFB Dryness Score (VAS) at Day 29.

Primary Efficacy Analyses:

The primary comparisons in this trial will be between CyclASol versus vehicle at Day 29. The primary efficacy endpoint (e.g. CFB in tCFS) will be summarized descriptively (n, mean, standard deviation, median, min, and max) and analyzed using an ANCOVA model with terms for baseline value and treatment.

Least squares mean for each treatment group and for the difference between treatment groups will be presented from the model together with two-sided p-values and 95% confidence intervals.

The primary analysis will use the Full Analysis Set (FAS) with available data per subject using Estimand 1, assuming the rate of missing data for an endpoint is <5%. If the rate of missing data for an endpoint is ≥5% and the missing data is:

1. Balanced between treatment groups, then the primary analysis will be based on the primary multiple imputation methodology as presented in Estimand 2 and the available data analyses will become robustness analyses.

2. Imbalanced between treatment groups, then the primary analysis will be based on trimmed means (Permutt et al., 2017) as presented in Estimand 3 and the available data analyses will become robustness analyses.

Two-sample t-tests, Wilcoxon rank sum tests and mixed-effect repeated measures analysis comparing treatment groups will be performed as sensitivity analyses.
Additional sensitivity analyses will include repeating the primary analysis on the FAS imputing missing data using multiple imputation methodology under different assumptions of missingness (at random and not at random); last observation carried forward (LOCF); trimmed means as specified in Estimand 3 ; and the per protocol set (PPS) with available data per subject. Sensitivity analyses will also include repeating the primary analysis using the FAS with multiple imputation methology and different assumptions for intercurrent events due to COVID-19.

Secondary Efficacy Analysis:
The following key secondary endpoints will be tested hierarchically:

1. CFB in total conjunctival lissamine green staining score (Oxford scale) at Day 29
2. Proportion of responders in cCFS score (≥ 1 score improvement on NEI scale) at Day 29
3. Proportion of responders in tCFS (≥ 3 score improvement on NEI scale) at Day 29
4. CFB in cCFS score (NEI scale) at Day 29

Inference will only be made on these endpoints, at a 2-sided alpha = 0.05, if both primary endpoints and any higher order key secondary endpoints are statistically significant at a 2-sided alpha = 0.05 in favor of CyclASol.

If all four of these key secondary endpoints and both primary endpoints demonstrate statistical significance at a 2-sided apha = 0.05 in favor of CyclASol, then the following two key secondary endpoints will be tested simultaneously using Hochberg's procedure to maintain an overall two-sided alpha = 0.05:

- CFB in visual analogue scale (VAS) for blurred vision and at Day 29
- CFB in tCFS (NEI scale) at Day 15

Quantitative key secondary efficacy endpoints will be analyzed (similar ANCOVA model) and summarized similarly to the primary effiacy endpoints. Dichotomous key secondary endpoints (proportion of responders) will be summarized descriptively (frequency and percentage) and analyzed using a logistic regression model with terms for baseline value and treatment.

Safety Variables
Adverse events (AEs) will be coded using the MedDRA dictionary. Frequencies and percentages of treatment-emergent adverse events (TEAEs) will be summarized at the subject level by system organ class and preferred term for all TEAEs, treatment related TEAEs, serious TEAEs, and TEAEs causing premature discontinuation by treatment group. Other safety endpoints including visual acuity, slit-lamp biomicroscopy findings, intraocular pressure, and dilated fundoscopy will be summarized by treatment group and visit using descriptive statistics.

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# LIST OF ABBREVIATIONS

| | |
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| AE | Adverse Event |
| AICc | Akaike Information Criterion with a correction for finite sample sizes |
| ANCOVA | Analysis of Covariance |
| BCVA | Best-Corrected Visual Acuity |
| BID | Twice Daily |
| BLQ | Below the Limit of Quantification |
| cCFS | Central corneal fluorescein staining |
| CFB | Change from Baseline |
| CFR | Code of Federal Regulations |
| CFS | Corneal fluorescein staining |
| CI | Confidence Interval |
| CRA | Clinical Research Associate |
| eCRF | Electronic Case Report Form |
| CRO | Contract Research Organization |
| CS | Compound Symmetry |
| CsA | Cyclosporine A |
| CV | Curriculum Vitae |
| DED | Dry Eye Disease |
| DEWS | (International) Dry Eye Workshop |
| ECG | Electrocardiography, Electrocardiogram |
| ET | Early Termination |
| ETDRS | Early Treatment of Diabetic Retinopathy Study |
| FAS | Full Analysis Set |
| FDA | Food and Drug Administration |
| g | Gram |
| GCP | Good Clinical Practice |
| GMP | Good Manufacturing Practice |
| HIPAA | Health Information Portability and Accountability Act |
| IB | Investigators' Brochure |
| ICH | International Conference on Harmonization |
| IEC | Independent Ethics Committee |
| IMP | Investigational Medicinal Product |
| IND | Investigational New Drug Application |
| IOP | Intraocular Pressure |
| IRB | Independent Review Board |
| IReST | International Reading Speed Texts |
| IRT | Interactive Response Technology |
| IUD | Intrauterine Device |
| LOCF | Last Observation Carried Forward |
| logMAR | Logarithm of the Minimum Angle of Resolution |
| MCMC | Markov Chain Monte Carlo |
| MedDRA | Medical Dictionary for Regulatory Activities |

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| MGD | Meibomian Gland Dysfunction |
| mL | Milliliter |
| µL | Microliter |
| mm | Millimeter |
| mmHg | Millimeters of Mercury |
| MNRead | The Minnesota Low-Vision Reading Test |
| NCS | Not Clinically Significant |
| NDA | New Drug Application |
| NEI | National Eye Institute |
| NONMEM | Non-linear mixed effects modeling |
| OD | Right Eye |
| OS | Left Eye |
| OSDI | Ocular Surface Disease Index |
| OU | Both Eyes |
| OTC | Over the Counter |
| PPS | Per Protocol Set |
| SAE | Serious Adverse Event |
| SAF | Safety Set |
| SFA | Semifluorinated Alkane |
| SOP | Standard Operating Procedures |
| tCFS | Total corneal fluorescein staining |
| TEAE | Treatment-emergent Adverse Event |
| TFBUT | Tear Film Break-up Time |
| TMF | Trial Master File |
| US(A) | United States (of America) |
| VA | Visual Acuity |
| VAS | Visual Analog Scale |
| WHO | World Health Organization |
| w/v | Weight per volume |
| w/w | Weight per weight |

# 2 INTRODUCTION

## 2.1 Dry Eye Disease (DED)

Dry Eye Disease (DED) is defined by the International Dry Eye Workshop (DEWS) 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 subjects visiting ophthalmic clinics report dry eye 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 dry eye 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 Rational

Cyclosporine A (CsA), a potent and selective immunomodulatory drug, acts as a regulator of T-cells via inhibition of calcineurin. 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. In the US, CsA eye drops formulated as an emulsion have been approved and marketed as Restasis 0.05% for this indication since 2002. In Europe, Ikervis™, a 0.1% CsA emulsion formulation, received a marketing authorization by the European Medicines Agency for the treatment of DED in early 2015. A second CsA containing product, Cequa™, a nano-emulsion was approved in the US in 2018.

CyclASol, in contrast, is a clear ophthalmic solution of CsA developed with the goal of avoiding the use of oils, surfactants and preservatives. Potential benefits from the CyclASol formulation include improved tolerability and efficacy, early onset of efficacy and decreased visual disturbances associated with oily eye drops, emulsions or ointments. Moreover, the multiple dose containers allow for a convenient handling.

For the solubilization of CsA in CyclASol, the semifluorinated alkane (SFA) perfluorobutylpentane (abbreviated F4H5 – this abbreviation indicates the number of carbon atoms that are fluorinated [4] and the number of non-fluorinated carbon atoms [5]) is used as the vehicle. The only other component in the formulation is ethanol as a manufacturing aid. F4H5 is colourless, has a high vapor pressure, and is immiscible with water while having nearly the same refractory index. F4H5's physical properties make it an optimal vehicle for topical ocular use. As a result of its high vapor pressure (Krafft et al. 2009), F4H5 dissipates quickly from the ocular surface and consequently does not interact physically with the tear film, as shown in rabbits, where F4H5 had no effect on tear film break-up time (TFBUT) (Agarwal et al. 2019). Due to the low surface tension of F4H5, CyclASol eye drops are of small size, with a volume of approximately 10 µL/drop, potentially reducing pre-corneal clearance. Importantly, the low surface tension of the CyclASol formulation facilitates dissemination of the applied eye drop on the conjunctiva. The dissemination and spreading properties are further thought to improve the local bioavailability.

## 2.3 Trial Rational

After successful preclinical testing and early clinical testing, a Phase 2b/3 clinical trial (CYS-003, ESSENCE) was recently completed confirming the effects seen in the Phase 2 clinical trial.

In CYS-003, CyclASol 0.1% met the primary sign endpoint, showing superiority compared to vehicle in change from baseline (CFB) in total corneal fluorescein staining (tCFS) at Day 29 with high statistical significance (p=0.0002). CyclASol 0.1% also showed trends of more improvement in the second primary endpoint of CFB in total OSDI© at Day 29. Additionally, a statistically significant greater improvement in the pre-specified secondary symptom endpoint CFB in "Dryness Score" (visual analogue scale for severity of dryness) at Day 29 was demonstrated in the CyclASol 0.1% treatment group compared to the vehicle group (p=0.0311).

Furthermore, the CyclASol 0.1% group consistently showed statistically significant improvements over time in corneal and conjunctival staining compared to vehicle. The clinical effects observed had an early onset after 14 days of treatment that continued over the 84-day treatment period.

This second pivotal Phase 3 trial is designed to replicate efficacy of CyclASol 0.1% BID dosing over vehicle as already demonstrated in CYS-003 (ESSENCE) on sign (CFB in tCFS) and symptom (CFB in Dryness Score) at Day 29. The trial will be powered to demonstrate efficacy of CyclASol 0.1% over vehicle in both endpoints. The trial will further investigate the safety and tolerability of CyclASol 0.1%.

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

The investigational medicinal product (IMP) CyclASol 0.1% Ophthalmic Solution contains CsA as active ingredient and its vehicle, which consists of F4H5. CsA is a potent and selective immunosuppressive drug, used routinely for decades as an oral immunomodulator in various indications. CsA containing eye drops have been proven safe over the last 15 years. The excipient F4H5 used in CyclASol is chemically inert, without pharmacologic activity and not undergoing metabolism in the human body. However, it is not part of any pharmaceutical product approved

so far. Therefore, it has been subjected to the same pivotal non-clinical studies as a new active substance with favorable results.

282 subjects have been exposed to CyclASol 0.05% or 0.1% for up to 4 months duration in clinical trials to date (clinical trials CYS-001: Phase 1; CYS-002: Phase 2; CYS-003: Phase 2b/3) with a favorable outcome in safety and tolerability.

In the Phase 1 clinical trial with CyclASol the most frequently reported TEAE was headache (1 subject [5.6%] after CyclASol and 3 subjects [16.7%] after vehicle) and in the Phase 2 clinical trial the most frequently reported ocular TEAE was visual acuity reduced (4 subjects [7.8%] after CyclASol 0.1% and 1 subject [1.9%] after vehicle). This was similar in the Phase 2b/3 clinical trial with 8 subjects (2.4%) who experienced reduced visual acuity, 5 subjects (3.1%) in the CyclASol 0.1% group and 3 subjects (1.8%) in the vehicle group. All trials showed a low incidence of instillation site reaction; there were none in the Phase 1 trial, one and 4 subject(s) on CyclASol 0.1% and 2 and 3 subjects on vehicle reported such events in Phase 2 and Phase 2/3, respectively.

In the Phase 1 clinical trial, there were no SAEs and no subject discontinued the study due to AEs.

In the Phase 2 clinical trial, three (3) subjects (1.4%) withdrew from the investigational medicinal product (IMP) due to an ocular TEAE (eye pain, chemical eye injury and conjunctivitis), two of which were considered possibly related to IMP but resolved by the end of the trial without sequelae. Three (3) treatment-emergent serious adverse events (SAEs) were reported during the Phase 2 trial. All SAEs were non-ocular, were considered not-related to IMP and had recovered by the end of the trial.

In the Phase 2/3 clinical trial, three subjects (0.9%) withdrew from study treatment due to a TEAE. All 3 TEAEs that led to withdrawal of treatment were ocular and occurred in the CyclASol 0.1% group, 2 of which, foreign body sensation and eyelid edema, were suspected by the investigator to be related to study drug and had recovered by the end of the study. The third event of ocular discomfort was not suspected to be related to study drug and had not recovered by the end of the trial. During the Phase 2b/3 trial three of the 328 randomized subjects (0.9%) experienced a SAE which were considered not-related to IMP. No deaths were observed in all three clinical trials.

Slit-lamp biomicroscopy and dilated fundoscopy assessments did not indicate any treatment-related clinically significant changes. Generally, none of the treatment groups experienced an appreciable change in mean visual acuity (VA) or in intra-ocular pressure (IOP) from baseline.

In both Phase 1 and 2 clinical trials, plasma concentrations of CyclASol were determined and found to be below the limit of quantification (BLQ) (i.e. <0.100 ng/mL) after application of 1 or 2 drops of CyclASol twice daily. Most samples showed F4H5 concentrations BLQ, sporadic samples with measurable F4H5 concentrations were hardly above the BLQ. Therefore, adverse effects resulting from systemic absorption of CsA and / or F4H5 appear very unlikely.

In summary, based on the preclinical and clinical data obtained to date, risks to subjects in the planned CYS-004 trial are considered very low. Furthermore, the subjects randomized in the trial will be closely monitored, and current standard ophthalmic safety assessments will be performed during the entire treatment period. The results demonstrated to date the efficacy of CyclASol on signs and symptoms of DED. Treatment with CyclASol therefore provides a favorable benefit-risk

profile in subjects with DED, addressing a clinical need that is currently not optimally addressed with available treatment options for DED.

This clinical trial CYS-004 is designed to confirm the positive benefit-risk profile.

# 3 TRIAL OBJECTIVES

## 3.1 Primary objective

- The primary objective of the study is to assess efficacy of CyclASol in comparison to the vehicle for the treatment of a sign and symptom of DED.

## 3.2 Secondary objectives

- The secondary objective is to assess safety and tolerability of CyclASol in comparison to the vehicle for the treatment of signs and symptoms of DED.
- Further objectives of the trial are to explore the effects of CyclASol on visual function related endpoints and to explore their correlation with corneal staining parameters.

# 4 TRIAL DESIGN

## 4.1 Overall Trial Design

This is a Phase 3 multicenter, randomized, double-masked, and vehicle-controlled clinical trial to evaluate the efficacy, safety and tolerability of CyclASol 0.1% Ophthalmic Solution in subjects with signs and symptoms of DED. Approximately 834 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 will be randomized at approximately 25 sites in the US to receive treatment with CyclASol or vehicle in a 1:1 ratio (approx. 417 subjects per treatment arm).

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

## 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, they should be performed in the respective order as listed. Trial Drug Dispensation is described in Section 7.1.5.

**Screening (Visit 0)**

All assessments required for the screening visit must be performed -14 (±2) days before the randomization, at baseline Visit 1. At least one eye, the same eye, must qualify with the following objective measures: tCFS score of ≥ 10 (NEI scale), lissamine green conjunctival score ≥ 2 (Oxford scale), and unanesthetized Schirmer's Test score between 1 mm and 10 mm inclusive. In addition, the subjects must have a Dryness Score ≥ 50 (VAS). Run-in medication and subject diary will be dispensed to qualified subjects.

**Baseline (Visit 1)**

On Day 1 (Visit 1), used and unused run-in medication and diary will be collected and checked for compliance. Eligible subjects will be evaluated for baseline signs and symptoms of dry eye disease (including reading assessments) and will be randomized to CyclASol or vehicle BID if they continue to meet all eligibility criteria. Subjects will be given a 14-day supply and will self-administer a single drop of the trial medication into each eye at the clinic. Subsequently the subject will be asked to complete a Drop Comfort Scale & Questionnaire. Each subject will be given a dosing diary to record when doses were taken. Study staff will help the subject to understand how to use the dosing diary and when the remaining doses should be taken.

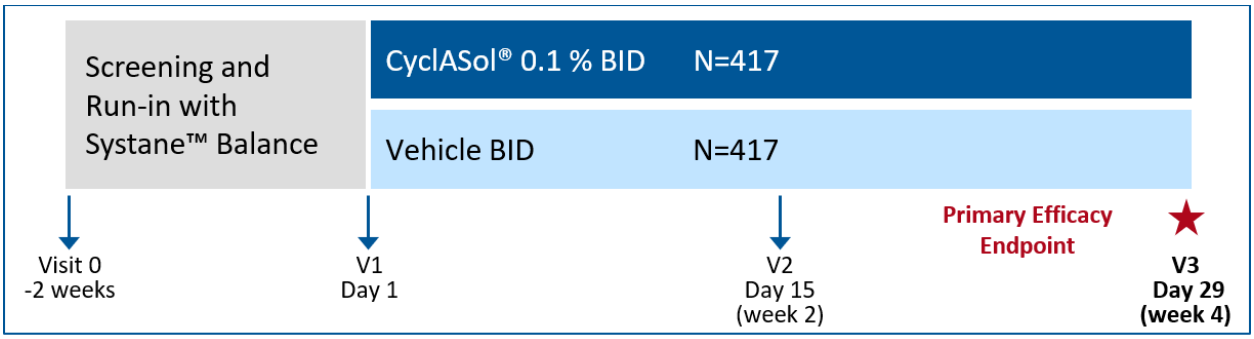
**Visits 2-3**

Subjects will return to the clinic on Day 15±2 (Visit 2) and 29±2 (Visit 3) to be evaluated for signs and symptoms of dry eye disease as well as safety. At Visit 2 the subject will be asked to complete the Drop Comfort Scale & Questionnaire. The subject will be asked to perform reading assessments and to complete the Eyedrop Acceptability Questionnaire during Visit 3. Used and unused trial medication should be returned to the clinic at both visits and a new trial medication bottle will be dispensed (at Visit 2). The dosing diary will be collected during each visit and checked for compliance. Subjects will be discharged from the trial after all Visit 3 assessments have been completed.

**Early Termination (ET)**

Subjects who terminate early during the treatment period will be asked to complete all assessments as indicated at Visit 3 on the schedule of assessments prior to commencement of any alternative dry eye therapy (if considered possible). Dosing diary and trial medication will be collected. Subjects who are terminated early from the trial will not be replaced.

## 4.4 Trial Flow




## 4.5 Enrollment and Treatment Assignment

All 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 Visits 0 and 1, each qualifying subject will then be randomized. Site and Dryness Score < 75 vs ≥ 75 (VAS) are used as stratification factors in this trial. The IRT will be used to account for the stratification factor while assigning the drug kit numbers at Visit 1. The subjects, investigators, [redacted] personnel involved in conduct and monitoring of the trial and sponsor will be masked to IMP assignment.

Each subject will spend approximately 6 weeks in the trial. The total duration of the trial from "first subject in" to "last subject out" is expected to be approximately 8 months.

## 4.6 Justification of Trial Design

This trial is designed as a confirmatory trial to the previously conducted Phase 2b/3 trial CYS-003 in subjects with DED.

The trial will be a randomized, double-masked, vehicle-controlled trial to demonstrate efficacy and safety of CyclASol after 1-month treatment duration. Vehicle controlled clinical trials are currently the standard for DED trials. Randomization and double masking are state of the art measures to reduce bias. Fast onset of efficacy is important for subjects and their compliance to therapies, thus early demonstration of efficacy (e.g. at 1 month) is desired and highly clinically relevant. Moreover, DED is a fluctuating condition with phase-like recurring dry eye complaints that may be linked to seasonal and / or environmental changes (van Setten et al. 2016), thus a primary endpoint assessment at a shorter treatment duration is considered clinically relevant and has a greater potential to capture the true drug effect.

Total corneal fluorescein staining (NEI grading) and Dryness Score (VAS) have been selected as the primary sign endpoint and primary symptom endpoint, respectively. Corneal staining is an accepted clinical endpoint in DED and a highly relevant marker in this disease, as stained areas represent punctate disruption and damage of the corneal epithelium (Pflugfelder et al. 2017).

Reduction of corneal staining reflects corneal surface healing and thus is highly representative of treatment success. In DED trials, common symptoms such as dryness, frequency of dryness, blurred vision are frequently graded using the VAS (Novack et al. 2017). Questionnaires using VAS to assess symptoms of DED are generally considered validated symptom questionnaires in DED.

The two primary endpoints, CFB in tCFS and CFB in Dryness Score, will be tested in a hierarchical order with tCFS being tested first. The hierarchical testing has been selected to protect the α – error.

### 4.7 Justification for Dose

The CyclASol 0.1% BID was selected for the first pivotal trial CYS-003 based on results of CYS-002. The aim of the current trial CYS-004 is to replicate the efficacy and safety results of CYS-003, consequently the same dose and dosing schedule will be tested.

## 5 TRIAL POPULATION

### 5.1 Number of Subjects (approximate)

An estimated 1800 subjects will be screened to randomly assign at least 834 subjects to IMP such that at least 379 evaluable subjects per treatment arm complete the trial.

### 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 dry eye in both eyes and meet all inclusion criteria and none of the exclusion criteria.

### 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 Dry Eye Disease in both eyes for at least 180 days before Visit 0;
4. 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;
5. Have a Dryness Score ≥ 50 (VAS) at Visit 0 and Visit 1;

6. 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;

7. 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;

8. Have an unanesthetized Schirmer's Test score between 1 mm and 10 mm inclusive at Visit 0 and Visit 1;

9. Have at least one eye, the same eye, satisfy inclusion criteria 6, 7, and 8; and

10. Be able and willing to follow instructions and participate 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**:**

1. 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;

2. 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;

3. Had Lipiflow procedures performed during the past 180 days prior to Visit 0;

4. Have abnormal lid anatomy (e.g. incomplete eyelid closure, entropion, or ectropion) or abnormal blinking;

5. 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);

6. Have an ocular or periocular malignancy;

7. Have a corneal epithelial defect, or have in more than 2 of the 5 corneal regions > 50% confluent corneal staining;

8. Have a history of herpetic keratitis;

9. Have active ocular allergies or ocular allergies that may become active during the trial period;

10. Have worn contact lenses within 90 days before Visit 0 or anticipate using contact lenses during the trial;

11. 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;

12. Be diagnosed with an ongoing ocular infection;

13. 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;

14. Have active ocular or periocular rosacea or a pterygium;

15. 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;

16. Have used topical CsA or Lifitegrast within 60 days before Visit 0;

17. Have used any topical antiglaucoma medications (including prostaglandin analogues applied to eyelid margin) within 90 days before Visit 0;

18. 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;

19. Have used any topical ocular or facial steroids, or serum tears or oral doxycycline, or oral tetracycline within 30 days before Visit 0;

20. 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;

21. 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;

22. 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;

23. Have been randomized in a previous CyclASol trial;

24. Be a woman who is pregnant, nursing, or planning a pregnancy;

25. 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);

26. 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;

27. Have an uncontrolled systemic disease;

28. Have a known allergy or sensitivity to the IMP or its components: Cyclosporine A (CsA) or semifluorinated alkanes (SFA);

29. 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

30. 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.

## 5.5 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 after randomization **from the trial** for any of the following reasons:

- Occurrence of pregnancy;
- Withdrawal of subject`s consent;
- Emergency unblinding has occurred.

A subject **must be** discontinued **from treatment** after randomization for any of the following reasons (but may remain in the trial for follow up assessments):

- 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 as judged by the investigator and/or sponsor;
- 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;

- 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 8.6.2 must be completed.

The trial **can be** prematurely discontinued as described in Section 8.7.

# 6 TRIAL PARAMETERS

## 6.1 Efficacy Endpoints

### 6.1.1 Primary Efficacy Endpoints

Two primary endpoints will be tested in the following order using hierarchical fixed sequence testing:

1. CFB in tCFS (NEI scale) at Day 29
2. CFB in Dryness Score (VAS) at Day 29

### 6.1.2 Key Secondary Efficacy Endpoints

The following key secondary efficacy endpoints will be evaluated:

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

### 6.1.3 Secondary Efficacy Endpoints

- 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

6.1.4 Other Prespecified Efficacy Endpoints

- 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
- Unanesthezied Schirmer's Test and CFB to each measured post-baseline visit
- Proportion of responders in unanesthetized Schirmer's Test (≥ 10 mm increase) at each measured post-baseline visit
- Tear Film Break-Up Time (TFBUT) and CFB at each measured post-baseline visit.

## 6.2 Safety Endpoints

- Visual acuity and CFB at each measured post-baseline visits;
- Slit-lamp biomicroscopy findings and CFB at each measured post-baseline visits;
- Intraocular pressure and CFB at each measured post-baseline visits;
- Dilated fundoscopy findings and CFB at each measured post-baseline visits;
- Systemic and ocular AEs

## 6.3 Other Endpoints

In addition to the efficacy and safety endpoints above, the comfort of the trial drug will be assessed using:

- Drop Comfort Scale and Questionnaire
- Eyedrop Acceptability Questionnaire

# 7 TRIAL MATERIALS

## 7.1 Investigational Medicinal Product(s)

### 7.1.1 IMP(s)/ Formulation(s)

Run-In

- Systane Balance Lubricant Eye Drops

Randomized IMPs

**Table 1. Active Investigational Medicinal Product**

| | **Investigational Product** |
|---|---|
| **Product name:** | CyclASol |
| **Chemical name:** | Cyclosporine A 0.1% (w/v) in perfluorobutylpentane |
| **Dosage form:** | 2 mL ophthalmic solution |
| **Unit dose:** | 9-11 µL drop size |
| **Route of administration:** | Topical ocular administration |
| **Physical description:** | Colorless and clear ophthalmic solution |
| **Excipients:** | Perfluorobutylpentane/1% (w/w) Ethanol |
| **Manufacturer:** | Alliance Medical Products, Inc., DBA Siegfried Irvine, 9342 Jeronimo Rd., Irvine, CA 92618, USA |

**Table 2. Control/Reference Investigational Medicinal Product**

| | **Control/Reference Investigational Product** |
|---|---|
| **Product name:** | Vehicle (F4H5) |
| **Chemical name:** | Perfluorobutylpentane |
| **Dosage form:** | 2 mL ophthalmic solution |
| **Unit dose:** | 9-11 µL drop size |
| **Route of administration:** | Topical ocular administration |
| **Physical description:** | Colorless and clear ophthalmic solution |
| **Excipients:** | 1% (w/w) Ethanol |
| **Manufacturer:** | Alliance Medical Products, Inc., DBA Siegfried Irvine, 9342 Jeronimo Rd., Irvine, CA 92618, USA |

### 7.1.2 Labeling and Packaging of IMP

For the run-in period, 1 bottle of Systane Balance will be labelled according to legal requirements.

IMP will be labelled according to the legal requirements and packaged into individual subject kits, each containing 2 bottles of CyclASol 0.1% Ophthalmic Solution or Vehicle. See Section 7.1.5 for details regarding dispensation to subjects. The subject should note the day of bottle opening 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
- Investigational new drug statement
- Lot 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. IMP must be stored at room temperature under temperature-monitored conditions and must not be refrigerated. The IMP must be protected from light. Subjects should be instructed to store IMP at room temperature and out of childrens' reach at home. Subjects should not use a dispensed bottle that has been opened for more than 30 days.

### 7.1.4 Administration of run-in medication

At the end of Visit 0, qualified subjects will receive the run-in medication (Systane Balance). Their first dose of run-in medication will be in-office. They will then be instructed to dose at home BID up to and including the morning of Visit 1 (at least 2 hours before first ophthalmic examination). Subjects will be instructed to dose in the morning and in the evening at bedtime. Subjects will record in the subject's diary that their doses were taken. At Visit 1, used/unused run-in will be collected from subjects.

### 7.1.5 IMP Dispensation

At the end of Visit 1, qualified subjects will be randomized, and a kit of IMP containing 2 bottles for each subject will be assigned using IRT. The subject will receive the first bottle out of the assigned subject kit. Subjects will be instructed on appropriate hygiene and eye drop dosing technique for multiple use drops by the site's staff and given written instructions. The first dose of IMP will be administered while being at the trial site: subjects will self-administer IMP eye drops under the supervision of the site staff. They will then be instructed to dose BID, in the morning and in the evening at bedtime, up to and including the morning of Visit 2 (at least 2 hours before first ophthalmic examination). Subjects will record in the subject's diary that their doses were taken.

At Visit 2, IMP will be collected from subjects for drug accountability. The subject will be dispensed the second bottle from the IMP kit to continue BID dosing. The second dose of IMP of this day will be administered while being at the trial site: subjects will self-administer IMP eye drops under the supervision of the site staff (at this day the potential evening dose shall not be used). The subject will be reminded to dose BID, in the morning and in the evening at bedtime, up to and including the morning of Visit 3 (at least 2 hours before first ophthalmic examination). Subjects will record in the subject's diary that their doses were taken.

In case the subject needs a replacement bottle of IMP, the next bottle from the kit will be dispensed. If no bottle remains in the kit, a new kit will be assigned to the subject using IRT.

At Visit 3 remaining IMP will be collected from subjects for drug accountability.

#### 7.1.6 Instructions for Use and Administration

Subjects will be instructed to instill one drop in each lower eyelid two times daily (in the morning and in the evening before bed). Subjects will be instructed to use a second drop only if the first drop misses the eye. Subjects will receive detailed written instructions how to store and dose IMP, and how to complete their diary.

Subjects will be instructed to immediately contact the site if there is any problem with the IMP or run-in medication (e.g. if the bottle was dropped or lost).

## 7.2 IMP Accountability

The investigator 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 investigator 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, dispensing records, and inventory forms will be examined and reconciled by designated site personnel. At each visit, subjects will return all bottles to designated site personnel for accountability purposes. Accountability will be ascertained by performing reconciliation between the amount of IMP cartons (kits and their components) sent to the site, the amount used and unused at the time of reconciliation. No investigative drugs 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 drug disposition forms to the sponsor after the completion of the trial.

## 7.4 Other Trial Supplies

Diaries, questionnaires, VAS rulers, Schirmer's test strips, sodium fluorescein, lissamine strips, Fluress, Tropicamide, Urine pregnancy tests.

# 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. Whenever possible, medications should be administered in dosages that remain constant throughout the study duration.

The use of any concurrent medication, prescription or over-the-counter, is to be recorded on the subject's source document and corresponding eCRF along with the reason the medication was taken.
Physical therapies such as lid scrubs, lid wipes (with the exception of hypochlorous acid wipes), warm compresses had to be stable within the last 30 days prior to Visit 1 and should be maintained stable throughout the trial.

## 8.2 Prohibited Medications

Disallowed medications and treatments are listed in the Exclusion Criteria (Section 5.4). All medications and treatments that were not allowed prior to the trial are 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) shall be used within 2 hours prior to V0 and throughout the course of the trial. Hypochlorous acid wipes are not allowed.

The Medical Monitor should be notified before prohibited medication or therapy is administered, unless the safety of the subject requires immediate action. The decision to administer a prohibited medication or therapy should be done with the safety of the subject as the primary consideration. In case such medication becomes necessary, a protocol deviation will be noted, however, subjects should continue the trial and dosing with IMP as long as the prohibited medication and or the reason for it is not considered a safety concern by the investigator or the medical monitor.

## 8.3 Restrictions and Prohibitions

Subjects will be asked to refrain from the following, for **24 hours** prior to their visits:

- Dangerous sport activities (e.g. skiing, mountain climbing, etc.).
- Challenging climates (e.g. smoking rooms, sauna, airplane etc.).

Subjects will be asked to refrain from the following for **12 hours** prior to their visits:

- Swimming in a chlorinated pool.

In case visual disturbances should occur upon instillation of the drop the subject is 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 performed as described in Appendix 2: Examination Procedures, Tests, Equipment, and Techniques.

**Visit 0 (Day -14 ± 2 days): Screening**

- Informed consent / HIPAA
- Medical/medication history and demographic data
- Review of qualification criteria (in-/exclusion criteria)
- Urine pregnancy test for females of childbearing potential
- Dryness Score (VAS)
- VAS for blurred vision, fluctuating vision, reading problems, looking at screens, driving at night, awareness of dry eye symptoms, frequency of dryness
- Ocular Surface Disease Index (OSDI score)
- Visual acuity (ETDRS)
- Slit-lamp biomicroscopy
- Tear Film Break-Up Time (TFBUT)
- Fluorescein staining (NEI scale)
- Lissamine green staining (Oxford scale)
- Minimum 15-minute wait from end of lissamine green staining to start of unanesthetized Schirmer's Test
- Unanesthetized Schirmer's Test
- Intraocular pressure
- Dilated fundoscopy
- In-office instillation of run-in drops to qualified subjects
- Dispensation of subject diary and run-in
- Non-leading AE questioning
- Qualified subjects are scheduled for Visit 1

**Visit 1 (Day 1): Baseline / Randomization**

- At the beginning of the visit the subject will be asked about the time of last dose. Assessments should not be done earlier than 2 hours after run-in instillation.
- Collection and review of run-in and diary
    - Calculate subject compliance as described in Section 8.5
    - Ask subject if he/she dosed with run-in the morning of Visit 1 and, if applicable, record the time of the dosing
- Medical/Medication history update/Non-leading AE questioning
- Review of qualification criteria
- Dryness Score (VAS)

- VAS for blurred vision, fluctuating vision, reading problems, looking at screens, driving at night, awareness of dry eye symptoms, frequency of dryness
- Ocular Surface Disease Index (OSDI score)
- Visual acuity (ETDRS)
- Slit-lamp biomicroscopy
- Tear Film Break-Up Time (TFBUT)
- Fluorescein staining (NEI scale)
- Lissamine green staining (Oxford scale)
- Reading assessments (MNread, sustained silent reading, IReST)
- Unanesthetized Schirmer's Test
- Randomization
- In-office instillation of randomized IMP
- Drop Comfort Scale and Questionnaire
- Dispensation of subject diary and randomized IMP
- Non-leading AE questioning
- Randomized subjects are scheduled for Visit 2

**Visit 2 (Day 15 ± 2): 2-Week Follow-Up**

- At the beginning of the visit the subject will be asked about the time of last dose. Assessments should not be done earlier than 2 hours after IMP instillation.
- Collection and review of IMP and subject diary
    - Calculate subject compliance with recording doses in the subject diary as described in Section 8.5
    - Ask subject if he/she dosed with IMP the morning of Visit 2 and, if applicable, record the time of the dose
    - Perform accountability on returned IMP and calculate dosing compliance as described in Section 8.5
- Medical/Medication history update/Non-leading AE questioning
- Dryness Score (VAS);
- VAS for blurred vision, fluctuating vision, reading problems, looking at screens, driving at night, awareness of dry eye symptoms, frequency of dryness
- Ocular Surface Disease Index (OSDI score)
- Visual acuity (ETDRS)
- Slit-lamp biomicroscopy
- Fluorescein staining (NEI scale)
- Lissamine green staining (Oxford scale)
- Dispensation of subject diary and randomized IMP
- In-office instillation of randomized IMP (2nd dose of the day)
- Drop Comfort Scale and Questionnaire
- Non-leading AE questioning
- Schedule for Visit 3

**Visit 3 (Day 29 ± 2): 4-Week Follow-Up and Trial Exit or ET**

- At the beginning of the visit the subject will be asked about the time of last dose. Assessments should not be done earlier than 2 hours after IMP instillation.
- Collection and review of IMP and subject diary;
  - Ask subject if he/she dosed with IMP the morning of Visit 3 and, if applicable, record the time of the dose
  - Perform accountability on returned IMP and calculate dosing compliance as described in Section 8.5
- Medical/Medication history update/Non-leading AE questioning
- Urine pregnancy test for females of childbearing potential
- Dryness Score (VAS)
- VAS for blurred vision, fluctuating vision, reading problems, looking at screens, driving at night, awareness of dry eye symptoms, frequency of dryness
- Ocular Surface Disease Index (OSDI score)
- Visual acuity (ETDRS)
- Slit-lamp biomicroscopy
- Tear Film Break-Up Time (TFBUT)
- Fluorescein staining (NEI scale)
- Lissamine green staining (Oxford scale)
- Reading assessments (MNread, sustained silent reading, IReST)
- Unanesthetized Schirmer's Test
- Intraocular pressure
- Dilated fundoscopy
- Eyedrop acceptability questionnaire
- Non-leading AE questioning
- Trial exit

### 8.4.2 Early Termination/Discontinuations

The following data from subjects discontinuing before randomization will be captured in the eCRF:

- Demographics
- AEs / SAEs
- Reason for discontinuation

Randomized and dosed subjects who discontinue treatment for any reason will be encouraged to undergo all subsequent trial visits and assessments.

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

If a randomized subject is discontinued from the trial before Visit 3 (Day 29 ± 2 days) and is not willing to perform all subsequent visits and assessments, all evaluations of Visit 3 (at least all

safety related evaluations) should be performed at Early Termination Visit on the day of early termination/discontinuation or at the discretion of the investigator.

#### 8.4.3 Unscheduled Visits

An unscheduled visit may be performed during the course of 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;
- Visual acuity;
- Intraocular pressure;
- Urine pregnancy test (for women of childbearing potential);
- Dilated fundoscopy;
- Assessment of AEs;
- Assessment of concomitant medications and/or treatments; and
- Any other assessments needed in the judgement of the investigator.

## 8.5 Compliance with Dosing/Protocol Deviations

Subjects will be instructed on the proper use and storage of the IMP at Visits 0, 1 and 2 and provided with written instructions. Subject diaries and IMP will be collected at each visit from Visit 1 up to and including Visit 3 to assess subject compliance with the protocol.

Subject dosing compliance will be determined by the subject's response or lack thereof to the prompt "Was the dose taken?" in the subject diary. If more than 20% of the responses to the total expected dose-taken prompts are checked "no," left blank, or missing, then the subject will be deemed noncompliant for dosing and a deviation recorded.

A protocol deviation is any noncompliance with the clinical study protocol, GCP, or Study Operations manual requirements. The noncompliance may be on the part of the subject, the PI, or study staff. The Investigator should not deviate from the requirements of this protocol without prior written approval of the Medical Monitor or Sponsor/CRO, with the exception of a medical emergency. As a result of deviations, corrective actions may need to be developed by the study staff and implemented promptly.

All deviations from the protocol must be addressed in study subject source documents and promptly reported to the IRB according to their requirements. Protocol deviations will be assessed before DB lock as major or minor.

In case of a positive SARS-CoV-2 (COVID-19) test result or infection the subject must not undergo visits in the clinic until he/she is considered not to be infectious anymore. Potential skipped or delayed visits must be recorded as protocol deviations and denoted to be COVID-19

related. For those subjects, a Trial Exit Visit - also at a later point - is recommended if their health state allows.

## 8.6 Subject Disposition

### 8.6.1 Completed Subjects

A completed subject is one who has not been discontinued from the trial.

### 8.6.2 Discontinued Subjects

Notification of a subject's discontinuation and the reason for discontinuation will be made to [redacted] and/or Novaliq 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

Discontinuations that are the direct result of SARS-CoV-2 (COVID-19) will be classified separately and clearly documented in the eCRF. A separate field will be completed to denote a COVID-19 related discontinuation and the specific reason will be documented utilizing the above noted list of subcategories.

Subjects must be discontinued as outlined in Section 5.5.

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

## 8.7 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.
- Poor enrollment of subjects making completion of the trial within an acceptable time frame unlikely.
- Discontinuation of development of the sponsor's IMP.
- 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.

## 8.8 Trial Duration

An individual subject's participation will involve four visits over approximately a 6-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 8 months.

Subjects who completed trial CYS-004 without major protocol deviations, have been compliant with CYS-004 trial procedures and application of IMP may be invited to enroll into an extension trial, CYS-005. CYS-005 will be a 12-months open-label safety extension trial of CYS-004 to assess the safety of topical CyclASol 0.1% for the treatment of DED.

# 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 drug-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 or the run-in medication, this should also be considered a new AE and reported. Any medical condition present prior to the administration of the IMP or the run-in medication 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 dry eye status of the subject exceeds their previous experiences with the condition. This will be determined by the subject and the investigator.

A clinically relevant worsening of visual acuity from screening visit (Visit 0), defined as an increase of 0.22 or greater in logMAR score, will be considered an AE.

IMP includes the investigational drug under evaluation and any comparator drug, vehicle, 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 signing informed consent 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. CyclASol contains the active ingredient CsA and the vehicle F4H5 and has been tested in three clinical studies up to now, and AEs of those have been listed in the Investigator's Brochure. Therefore, the following definition will be used:

- *Unexpected*: An AE that is not listed in the investigator's brochure (IB) in the Adverse Reaction Section at the specificity or severity that has been observed.
- *Expected*: An AE that is listed in the investigator's brochure (IB) 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
- Lost to Follow-up
- 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 patient gives informed consent until the end of the Visit 3 at (Day 29 ± 2) or Early Termination.

If the investigator detects a Serious Adverse Event (see Section 9.2) in a study patient 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 or 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 serious adverse event (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 AEs and their outcomes must be reported to [redacted] Novaliq, [redacted] (external safety service provider) 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] 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] 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] 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:

| | |
|---|---|
| External safety service provider [redacted] | |
| Email: | [redacted] |
| Fax: | [redacted] |

Contact information for Medical Monitor:

| | |
|---|---|
| Medical Monitor: | [redacted] |
| Office Telephone: | [redacted] |

| | |
|---|---|
| Alternative Telephone (24-hr line): | [redacted] |
| Fax: | [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/IEC as required by the IRB/IEC, 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. When medically necessary, the investigator may need to determine what treatment arm has been assigned to a subject.

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's external safety provider 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/or [redacted] and Novaliq. 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 both the Early Termination and Follow-up 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/ faxed 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 Fax number (see Section 9.3). [redacted] will forward the documentation to the medical monitor, [redacted] project manager and the sponsor for review.

At the completion of the pregnancy, the Pregnancy Outcome Form is to be submitted to [redacted] 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

The following analysis population will be considered:

- Full Analysis Set (FAS) – The FAS includes all randomized subjects who received at least one dose of investigation product. The primary analysis will be performed on the FAS. Subjects in the FAS will be analyzed as randomized.
- Per Protocol Set (PPS) – The PPS includes subjects in the FAS who do not have major protocol deviations and who complete the trial. Protocol deviations will be assessed prior to database lock and unmasking. The PPS will be analyzed using observed data only for efficacy variables. Subjects in the PPS will be analyzed as treated.
- Safety Set (SAF) – The SAF includes all randomized subjects who have received at least one dose of the investigational product. The SAF will be analyzed for all safety assessments. Subjects in the SAF will be analyzed as treated.

The statistical analysis of safety data will be performed for the SAF. The analysis of baseline and efficacy data will be performed for the FAS. The primary efficacy analyses will also be performed on the PPS as sensitivity analyses.

## 10.2 Statistical Hypotheses

The primary endpoints will be tested in a hierarchical fixed sequence in the following order.
The statistical hypotheses for the primary endpoint of CFB in tCFS score (NEI scale) at Day 29 are as follows:

$H_{01}$: The difference, between study eyes treated with CyclASol and study eyes treated with vehicle, in the mean CFB in tCFS score (NEI scale) at Day 29 = 0.

$H_{A1}$: The difference, between study eyes treated with CyclASol and study eyes treated with vehicle, in the mean CFB in tCFS score (NEI scale) at Day 29 ≠ 0, with superiority claimed if the difference is less than 0 (CyclASol minus vehicle).

The statistical hypotheses for the primary endpoint of the CFB in Dryness Score (VAS) at Day 29 are as follows:

$H_{02}$: The difference, between subjects treated with CyclASol and subjects treated with vehicle, in the mean CFB in Dryness Score (VAS) at Day 29 = 0.

$H_{A2}$: The difference, between subjects treated with CyclASol and subjects treated with vehicle, in the mean CFB in Dryness Score (VAS) at Day 29 ≠ 0, with superiority claimed if the difference is less than 0 (CyclASol minus vehicle)

Hierarchical fixed sequence testing will be employed to maintain the type I error rate. The primary analyses will first test the difference in the mean CFB in tCFS score (NEI scale) between

treatments at Day 29. If the test of the difference is statistically significant at the two-sided alpha = 0.05 level in favor of CyclASol, then the trial will be considered a success; CyclASol will be declared to be superior to vehicle in the mean CFB in tCFS score (NEI scale) at Day 29; and the difference in the mean CFB in Dryness Score (VAS) between treatments at Day 29 will be tested at the two-sided alpha = 0.05 level.

If in addition to a statistically significant test of the difference in the mean CFB in tCFS score (NEI scale) at Day 29 in favor of CyclASol, the test of the difference in the mean CFB in Dryness Score (VAS) at Day 29 is also statistically significant in favor of CyclASol, then CyclASol will be declared to be superior to vehicle in both the mean CFB in tCFS score (NEI scale) and the mean CFB in Dryness Score (VAS) at Day 29.

## 10.3 Sample Size

The trial has been designed to have 90% power to reject both $H_{01}$ and $H_{02}$ assuming independence between the sign and symptom endpoint; positive correlation between these two endpoints would increase the overall power.

Three hundred seventy nine (379) full analysis set (FAS) subjects (study eyes) per treatment group yield 96.8% power to reject $H_{01}$ in favor of $H_{A1}$ and conclude superiority of CyclASol over vehicle in the mean CFB in tCFS score at Day 29 assuming a true difference (CyclASol minus vehicle) of -0.75, a common standard deviation of 2.7, and a two-sided alpha = 0.05.

Additionally, 379 FAS subjects per treatment group yield 93.0% power to reject $H_{02}$ in favor of $H_{A2}$ and conclude superiority of CyclASol over vehicle in the mean CFB in Dryness Score (VAS) at Day 29, assuming a true difference (CyclASol minus vehicle) of -5.0, a common standard deviation of 20.0, and a two-sided alpha = 0.05.

Therefore, assuming independence between tCFS score and Dryness Score (VAS), 379 FAS subjects per treatment group yields 96.8% * 93.0% = 90.0% power to reject both $H_{01}$ and $H_{02}$.

Accounting for subject discontinuations, approximately 834 total subjects (417 per treatment arm) will be enrolled assuming a dropout rate of approximately 10%. The sponsor may elect to enroll additional subjects to account for a dropout rate that exceeds 10%.

## 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 group. Summaries will be 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 last non-missing measure prior to the initiation of randomized study treatment. CFB will be calculated as Visit – Baseline. All primary and secondary analyses will be two-sided at a significance level of 0.05.

For observed measures obtained on Visit 3 more than + 7 days out of window for any reason, measures will be considered missing data for all efficacy analyses and not used for analyses unless otherwise specified.

### 10.4.2 Unit of Analysis

For efficacy endpoints, the unit of analysis will be the "study eye" as defined by the following:

Eyes are eligible for analysis if they meet all of the inclusion criteria. In the case that both eyes are eligible for analysis, the study eye will be the eye with higher mean tCFS score (NEI Scale) at Visit 1. If the mean tCFS score (NEI Scale) is the same in both eyes, then the right eye will be selected as the study eye.

### 10.4.3 Missing Data

The primary analysis will be completed with available data per subject from the Full Analysis Set (FAS) using Estimand 1, assuming the rate of missing data for an endpoint is <5%. Subjects that are inferred missing due to out of window visits greater than + 7 days will be considered missing for the purposes of comparison of the rate of missing data for an endpoint and balance of missing data between treatment groups. If the rate of missing data for an endpoint is ≥5% and the missing data is:

1. Balanced between treatment groups, then the primary analysis will be based on the primary multiple imputation methodology as presented in Estimand 2 and the available data analyses will become robustness analyses.
2. Imbalanced between treatment groups, then the primary analysis will be based on the trimmed means (Permutt et al., 2017) as presented in Estimand 3 and the available data analyses will become robustness analyses.

Additional sensitivity analyses will include repeating the primary analysis on 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) each using 30 imputed values; the FAS imputing missing data using last observation carried forward (LOCF); the FAS using trimmed means as specified in Estimand 3; and the PPS with available data per subject. Sensitivity analyses will also include repeating the primary analysis using the FAS with multiple imputation methology and different assumptions for intercurrent events due to COVID-19.

The imputation model, for each visit, under the assumption of missing **at** random is:

```
PROC MI DATA=INDATA SEED=97656 OUT=OUTDATA1 MINIMUM = 0 MAXIMUM = 3 ROUND = 1
NIMPUTE=30;
  MCMC INITIAL=EM;
```

```
 BY <TREATMENT>;
 VAR <BASELINE> <PARAMETER>;
RUN;
```

where PARAMETER has 5 levels and refers to the CFS score for each region (inferior, superior, central, temporal, and nasal).

The imputation model, for each visit, under the assumption of missing **not at** random is:

```
PROC MI DATA=INDATA SEED=36797 OUT=MDATA MINIMUM = 0 MAXIMUM = 3 ROUND = 1
NIMPUTE=30;
 MCMC IMPUTE=MONOTONE;
 VAR <BASELINE> <PARAMETER>;
RUN;

PROC MI DATA=MDATA SEED=38549 OUT=OUTDATA2 MINIMUM = . 0 0
          MAXIMUM = . 3 3 ROUND = . 1 1;
 CLASS <TREATMENT>;
 MONOTONE REG(<PARAMETER> = <BASELINE> / DETAILS);
 MNAR MODEL(<PARAMETER> / MODELOBS=(<TREATMENT>=' 'Vehicle''));
 VAR <TREATMENT> <BASELINE> <PARAMETER>;
RUN;
```

where PARAMETER has 5 levels and refers to the CFS score for each region (inferior, superior, central, temporal, and nasal).

### 10.4.4 Multiplicity Considerations

Hierarchical sequence testing will be employed to maintain the type I error rate. The primary analyses will first test the difference in the mean CFB in tCFS score (NEI scale) between treatments at Day 29. If the test of the difference is statistically significant at the two-sided alpha = 0.05 level in favor of CyclASol, then the trial will be considered a success; CyclASol will be declared to be superior to vehicle in the mean CFB in tCFS score (NEI scale) at Day 29; and the difference in the mean CFB in Dryness Score (VAS) between treatments at Day 29 will be tested at the two-sided alpha = 0.05 level.

If in addition to a statistically significant test of the difference in the mean CFB in tCFS score (NEI scale) at Day 29 in favor of CyclASol, the test of the difference in the mean CFB in Dryness Score (VAS) at Day 29 is also statistically significant in favor of CyclASol, then CyclASol will be declared to be superior to vehicle in both the mean CFB in tCFS score (NEI scale) and the mean CFB in Dryness Score (VAS) at Day 29.

Additionally, the key secondary efficacy endpoints will be alpha adjusted using the following composite methodology:

The following key secondary endpoints will be tested hierarchically:

1. CFB in total conjunctival lissamine green staining score (Oxford scale) at Day 29
2. Proportion of responders in cCFS score (≥ 1 score improvement on NEI scale) at Day 29)
3. Proportion of responders in tCFS (≥ 3 score improvement on NEI scale) at Day 29
4. CFB in cCFS score (NEI scale) to Day 29

Inference will only be made on these endpoints, at a 2-sided alpha = 0.05, if both primary endpoints and any higher order key secondary endpoints are statistically significant at a 2-sided alpha = 0.05 in favor of CyclASol.

If all four of these key secondary endpoints and both primary endpoints demonstrate statistical significance at a 2-sided apha = 0.05 in favor of CyclASol, then the following two key secondary endpoints will be tested simultaneously using Hochberg's procedure to maintain an overall two-sided alpha = 0.05:

- CFB in visual analogue scale (VAS) for blurred vision and at Day 29
- CFB in tCFS (NEI scale) at Day 15

Hochberg's procedure will be implemented by ordering the two-sided p-values from these three key secondary endpoints from highest to lowest. Each of these ordered p-values will be tested against 0.05 * [(3 - k) / 2] where 2 is the number of key secondary endpoints tested, 3 is the number of key secondary endpoints + 1, and k is the rank order of the endpoint. If the first ordered endpoint (highest to lowest) has a two-sided p-value < 0.05 in favor of CyclASol then both endpoints will be considered statistically significant. If the first ordered endpoint does not have a two-sided p-value < 0.05 in favor of CyclASol, then the two-sided p-value for the second ordered endpoint will be compared against 0.025.

### 10.4.5 Primary Efficacy Analyses

The primary comparisons in this trial will be between CyclASol versus vehicle at Day 29 in the FAS with available data per subject using the following estimand:

Estimand 1:

- Population: subjects with DED defined through enrollment criteria
- Endpoint:
  - CFB in tCFS in the study eye at Day 29
  - CFB of Dryness Score (VAS) at Day 29
- Intercurrent event:
  - Discontinuation of study medications is ignored, measures obtained after discontinuation of study medication will be analyzed. [treatment policy strategy]
  - Non-optimal compliance is ignored, measures will be analyzed regardless of treatment compliance. [treatment policy strategy]
  - Visits out of window by more than + 7 days, measures obtained will not be used for analysis and measures will not be imputed. [hypothetical strategy]
  - Withdrawal or missing data due to any reason. Missing data not imputed. [hypothetical strategy]
- Population-level summary:
  - Difference in the mean CFB in tCFS in the study eye at Day 29 between CyclASol and vehicle.
  - Difference in the mean CFB of Dryness Score (VAS) at Day 29 between CyclASol and vehicle.

Analyses based on Estimand 2 and Estimand 3 will be additional sensitivity analyses.

If the rate of missing data for an endpoint is ≥5% and the missing data is balanced between treatment groups, then the primary analysis will be based on multiple imputation methodology using the following estimand:

Estimand 2:

- Population: subjects with DED defined through enrollment criteria
- Endpoint:
  - CFB in tCFS in the study eye at Day 29
  - CFB of Dryness Score (VAS) at Day 29
- Intercurrent event:
  - Discontinuation of study medications is ignored, measures obtained after discontinuation of study medication will be analyzed. [treatment policy strategy]
  - Non-optimal compliance is ignored, measures will be analyzed regardless of treatment compliance. [treatment policy strategy]
  - Visits out of window by more than + 7 days, measures obtained will not be used for analysis and multiple imputations using randomized treatment-based Markov Chain Monte Carlo is used to impute data. [hypothetical strategy]
  - Withdrawal due to lack of efficacy or adverse events. Multiple imputations using vehicle treatment group-based Markov Chain Monte Carlo, regardless of randomized treatment group is used to impute missing data. [hypothetical strategy]
  - Missing data without withdrawal or withdrawal due to reasons other than lack of efficacy or adverse events. Multiple imputations using randomized treatment-based Markov Chain Monte Carlo is used to impute missing data. [hypothetical strategy]
- Population-level summary:
  - Difference in the mean CFB in tCFS in the study eye at Day 29 between CyclASol and vehicle.
  - Difference in the mean CFB in Dryness Score (VAS) at Day 29 between CyclASol and vehicle.

and analyses based on Estimand 1 and 3 will become sensitivity analyses.

If the rate of missing data for an endpoint is ≥5% and the missing data is imbalanced between treatment groups, then the primary analysis will be based on applying the trimmed means methodology (including permutation testing) to the following estimand:

Estimand 3

- Population: subjects with DED defined through enrollment criteria
- Endpoint:
  - CFB in tCFS in the study eye at Day 29
  - CFB of Dryness Score (VAS) at Day 29
- Intercurrent event:

  - Discontinuation of study medications is ignored, measures obtained after discontinuation of study medication will be analyzed. [treatment policy strategy]
  - Non-optimal compliance is ignored, measures will be analyzed regardless of treatment compliance. [treatment policy strategy]
  - Visits out of window by more than + 7 days. Measures obtained will not be used for analysis and single imputations using last observation carried forward to impute missing data. [hypothetical strategy]
  - Withdrawal due to lack of efficacy or adverse events. Single imputation using worst observation within the treatment group to impute missing data. [hypothetical strategy]
  - Missing data without withdrawal or withdrawal due to reasons other than lack of efficacy or adverse events. Single imputations using last observation carried forward to impute missing data. [hypothetical strategy]
- Population-level summary:
  - Difference in the trimmed mean CFB in tCFS in the study eye at Day 29 between CyclASol and vehicle.
  - Difference in the trimmed mean CFB in Dryness Score (VAS) at Day 29 between CyclASol and vehicle.

and analyses based on Estimand 1 and 2 will become additional sensitivity analyses.

If the rate of missing data for an endpoint is ≥5% then the determination of whether the missing data is balanced or imbalanced between treatment groups will be made based on a 2-sided alpha = 0.05 Pearson chi-squared test.

The proportion of data trimmed in the trimmed means analysis will be dependent on the maximum rate of missing data between treatment groups using the following:

| Maximum Rate of Missing Data (%) | Data Trimmed (%) |
|---|---|
| < 10% | 10% |
| 10% to <20% | 20% |

The primary efficacy endpoints (e.g. CFB in tCFS score [NEI scale] and Dryness Score [VAS]) will be analyzed separately using an ANCOVA model with terms for baseline value and treatment.

Least squares mean for each treatment group and for the difference between treatment groups will be presented from the model together with two-sided p-values and 95% confidence intervals.

Two-sample t-tests and Wilcoxon rank sum tests comparing treatment groups will be performed as sensitivity analyses. A mixed-effect repeated measures model will also be used as an additional sensitivity analysis of mean scores and mean changes from baseline at each visit. This model will include treatment, visit, and the interaction between treatment and visit as fixed effects, and subject as a random effect. An unstructured covariance matrix will initially be used to model the covariance among repeated measures; however, if the model fails to converge using this covariance structure, either heterogeneous TOEPLITZ, homogeneous TOEPLITZ, or CS

(compound symmetry) will be implemented according to the Akaike information criterion with a correction for finite sample sizes (AICc).

### 10.4.6 Key Secondary Efficacy Analyses

The following key secondary endpoints will be tested hierarchically:

1. CFB in total conjunctival lissamine green staining score (Oxford scale) at Day 29
2. Proportion of responders in cCFS score (≥ 1 score improvement on NEI scale) at Day 29)
3. Proportion of responders in tCFS score (≥ 3 score improvement on NEI scale) at Day 29
4. CFB in cCFS score (NEI scale) to Day 29

Inference will only be made on these endpoints, at a 2-sided alpha = 0.05, if both primary endpoints and any higher order key secondary endpoints are statistically significant at a 2-sided alpha = 0.05 in favor of CyclASol.

If all four of these key secondary endpoints and both primary endpoints demonstrate statistical significance at a 2-sided apha = 0.05 in favor of CyclASol, then the following two key secondary endpoints will be tested simultaneously using Hochberg's procedure (Section 10.4.4) to maintain an overall two-sided alpha = 0.05:

- CFB in VAS for blurred vision and at Day 29
- CFB in tCFS (NEI scale) at Day 15

Quantitative key secondary efficacy endpoints will be summarized similarly to the primary effiacy endpoints.

Dichotomous key secondary endpoints (proportion of responders) will be summarized descriptively (frequency and percentage) and analyzed using a logistic regression model with terms for baseline value and treatment. Least squares mean for each treatment group and for the difference between treatment groups will be presented from the model together with two-sided p-values and 95% confidence intervals. Pearson chi-squared analysis comparing the treatment groups will be performed as sensitivity analyses.

The primary analysis of the key secondary endpoints will use the FAS with Estimand 1, assuming the rate of missing data for an endpoint is <5% and analyses based on Estimands 2 and 3 will be sensitivity analyses. If the rate of missing data is ≥5%, then the primary analysis of the key secondary endpoints will be based on imputation methodology as defined Section 10.4.5 (Estimands 2 and 3) and the analyses based on other estimands will become sensitivity analyses.

In general, for the dichotomous endpoints imputations will be made according to the continuous endpoint estimands as described above, with the results then dichotomized.

If the rate of missing data for an endpoint is ≥5% and the missing data is imbalanced between treatment groups, then the primary analysis will be based on applying the imputations as specified for the continuous data under this scenario, with the exception that:

- Withdrawal due to lack of efficacy or adverse events. Missing data will be singly imputed as failure. [hypothetical strategy]

Trimmed mean analyses will not be applied to dichotomous endpoints.

Additional sensitivity analyses will include repeating the primary analysis on 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) each using 30 imputed values; the FAS imputing missing data using last observation carried forward (LOCF); the per protocol set (PPS) with available data per subject.

### 10.4.7 Secondary Efficacy Analyses

The primary analysis of the secondary endpoints will use the FAS with available data per subject.

Quantitative secondary efficacy variables will be summarized descriptively (n, mean, standard deviation, median, min, and max) and analyzed similarly to the primary endpoint at each measured visit and CFB. Least squares mean for each treatment group and for the difference between treatment groups will be presented from the model together with two-sided p-values and 95% confidence intervals.

Two-sample t-tests, Wilcoxon rank sum tests and mixed-effect repeated measures analysis will also be presented as sensitivity analyses.

Dichotomous secondary efficacy variables will be summarized descriptively (frequency and percentage) and analyzed similarly to the key secondary endpoints at each measured visit.

Least squares mean for each treatment group and for the difference between treatment groups will be presented from the model together with two-sided p-values and 95% confidence intervals. Pearson chi-squared analysis comparing the treatment groups will be performed as sensitivity analyses.

### 10.4.8 Exploratory analyses

Other prespecified efficacy and other endpoints will be summarized and analyzed similar to secondary endpoints.

### 10.4.9 Safety Variables Analyses

All safety analyses will be performed on the Safety Population.

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

Adverse events (AEs) will be coded using the MedDRA dictionary. Frequencies and percentages of treatment-emergent adverse events (TEAEs) will be summarized at the subject level by system organ class and preferred term for all TEAEs, treatment related TEAEs, serious TEAEs, and TEAEs causing premature discontinuation by treatment group. An AE is treatment emergent if it occurs or worsens after the first dose of trial treatment. Similar summaries will be presented for all TEAEs by maximal severity. 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.

Other safety endpoints including visual acuity, slit lamp biomicroscopy, intraocular pressure (IOP) and dilated fundoscopy will be summarized by treatment group and visit using descriptive statistics. Changes or shifts from baseline will also be summarized where appropriate. For assessments performed by eye, study eye and fellow eye will be summarized separately.
In addition, changes from baseline to worst on-treatment value for ocular safety assessments will be summarized.

#### 10.4.10 Interim Analyses

No interim analyses are planned for this trial.

## 10.5 Additional Analyses

In order to explore the effects of CyclASol on visual function related endpoints and the correlation with corneal staining parameters as outlined in section 3.2, additional analyses will be completed to comprehensively evaluate the relationship between tCFS and cCFS with reading efficacy measures (parameters of visual function), including correlation analysis at baseline and post-baseline (including CFB) using continuous measures. Continuous CFB measures in reading efficacy measures will also be summarized and analyzed by various categories of changes from baseline in CFS measures (e.g. 1, 2, 3 units in tCFS or 1.0 unit in cCFS). Evaluations of CFB will additionally account for baseline to determine if changes from baseline are constant or vary by a subject's starting value.

A complete write-up of the evaluation of the relationship between tCFS and cCFS and reading efficacy measures will be provided in a second statistical analysis plan, separate from the formal study statistical analysis plan and will be reported separately.

Additional analysis of study measures, combining / pooling data with the extension trial CYS-005 and / or the CYS-003 trial will be described in a third statistical analysis plan, separate from the formal study statistical analysis plan.

The primary and key secondary efficacy variables may be evaluated via a mixed effect modelling approach, potentially in combination with CYS-005 (open-label extension trial). Response variables will be modeled as a function of CyclASol or vehicle administration over time using linear mixed-effects modelling in NONMEM. The focus of this analysis will be to evaluate the

time-dependent CFB of the various efficacy measures in the vehicle and CyclASol treatment group. A covariate analysis will be conducted to identify subjects prone to respond to treatment. This analysis will use the FAS with all available data per subject.

A complete description of the planned analysis will be provided in a separate statistical analysis plan and will be reported separately.

# 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.

### 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 [redacted] 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 such as X-rays, laboratory tests, and ECGs. 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 source documents which will be transcribed 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 and randomized 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. For screen failure subjects, the following data will be entered: AE, SAE, demographics and reason for screen failure. 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, compact discs (CDs) containing copies of all applicable subjects' eCRFs will be provided 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 make routine site visits to review protocol compliance, assess IMP accountability, and ensure 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, [redacted] 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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