CONFIDENTIAL

Clinical Study Phase 3 Protocol
OPI-NYXDLD-302
LYNX-2

RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-MASKED STUDY OF THE SAFETY AND EFFICACY OF POS (0.75% PHENTOLAMINE OPHTHALMIC SOLUTION) IN SUBJECTS WHO HAVE PREVIOUSLY HAD KERATOREFRACTIVE SURGERY AND HAVE DECREASED VISUAL ACUITY UNDER MESOPIC CONDITIONS

Original: **February 1, 2024**

Amendment 1 (v2.0) **July 11, 2025**

CONFIDENTIAL
This document contains Opus Genetics, Inc. (Opus) information that is confidential, a trade secret, and/or proprietary in nature. It is loaned to you for your confidential use on behalf of Opus and is not to be photocopied, disclosed, or transmitted to any other person or party who is not covered by a Confidential Disclosure Agreement with Opus. As the Principal Investigator, you are responsible for the safekeeping and return of this document to Opus upon request. You will be sent updated information and/or amendments as they become available.

SPONSOR SIGNATURE & CONTACTS

Study Title:	Randomized, Placebo-Controlled, Double-Masked Study of the Safety and Efficacy of POS (0.75% Phentolamine Ophthalmic Solution) in Subjects Who Have Previously Had Keratorefractive Surgery and Have Decreased Visual Acuity Under Mesopic Conditions
Study Number:	OPI-NYXDLD-302
Original Protocol:	February 1, 2024
Amendment 1:	July 11, 2025

Person authorized to sign the protocol and protocol amendment(s) for the Sponsor, Opus Genetics, Inc.

[redacted]

Signature

Date

[redacted]

Signature

Date

INVESTIGATOR'S AGREEMENT

OPI-NYXDLD-302
LYNX-2

RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-MASKED STUDY OF THE SAFETY AND EFFICACY OF POS (0.75% PHENTOLAMINE OPHTHALMIC SOLUTION) IN SUBJECTS WHO HAVE PREVIOUSLY HAD KERATOREFRACTIVE SURGERY AND HAVE DECREASED VISUAL ACUITY UNDER MESOPIC CONDITIONS

Original Protocol: **February 1, 2024**
Amendment 1: **July 11, 2025**

Investigator Agreement:

I, the undersigned, have reviewed this protocol and I agree to conduct this protocol in accordance with Good Clinical Practice, the ethical principles set forth in the Declaration of Helsinki, and with the U.S. Code of Federal Regulations governing electronic records; electronic signatures (21 CFR 11), the protection of human subjects (21 CFR 50), financial disclosure by Clinical Investigators (21 CFR 54), Institutional Review Boards (21 CFR 56), and the obligations of Clinical Investigators (21 CFR 312).

Signature: _____ Date: _____

Printed Name: _____

PROCEDURES IN CASE OF EMERGENCY

EMERGENCY CONTACT INFORMATION

Role in Study	Name	Contact Information
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

1 STUDY SUMMARY

Study Number	**OPI-NYXDLD-302**
Clinical Phase	Phase 3
Type of Study	Randomized, placebo-controlled, double-masked study of the safety and efficacy of POS (0.75% Phentolamine Ophthalmic Solution) in subjects who have previously had keratorefractive surgery and have decreased visual acuity (VA) under mesopic conditions
Name of Investigational Product	POS Eye Drops – 0.75% Phentolamine Ophthalmic Solution
Duration of Study	Approximately 6 weeks, with an option to continue for an additional 42 weeks
Rationale	Individuals who have had prior refractive surgeries (laser-assisted in situ keratomileusis [LASIK], photorefractive keratectomy [PRK], small-incision lenticule extraction [SMILE], radial keratotomy [RK], etc.) may experience visual disturbances, including glare, halos, and/or starbursts (a series of concentric rays or fine filaments radiating from bright lights), under mesopic conditions (low light). When the pupil naturally dilates in these subjects with highly aberrated corneas, individuals may experience a loss of mesopic low-contrast visual acuity (mLCVA) and a loss of contrast sensitivity. It is proposed that a moderate pharmacologic reduction of pupil size (20–25%) by the application of an alpha-1 antagonist has the potential to mitigate these visual disturbances in many affected individuals. Phentolamine is a non-selective alpha-1 and alpha-2 adrenergic antagonist acting on the adrenergic receptors and inhibiting contraction of the smooth iris dilator muscle. When topically applied, phentolamine inhibits contraction of the iris dilator muscle, resulting in a smaller pupil size. Previous Phase 2 clinical trials showed that POS significantly decreases pupil diameter (PD) and improves mLCVA in subjects with post−refractive surgery visual disturbances after single- and multiple-dose (14 days) treatment. In these trials, POS was generally well tolerated in the eye (the most common complaint was transient mild-to-moderate ocular

	hyperemia and eye irritation), and there were no clinically meaningful systemic effects, as measured by heart rate (HR) and blood pressure (BP).
Study Objectives	The primary objectives of this study are: • To evaluate the efficacy of POS to improve mLCVA in subjects with post−refractive surgery visual disturbances • To evaluate the efficacy of POS to improve VA under various lighting conditions in subjects with post−refractive surgery visual disturbances • To evaluate tachyphylaxis of the therapeutic response to POS in subjects with post−refractive surgery visual disturbances • To evaluate the safety of POS in subjects with post−refractive surgery visual disturbances
Design	This is a randomized, placebo-controlled, double-masked, 2-stage, multiple-dose, multicenter, Phase 3 study in approximately 200 subjects with post−refractive surgery visual disturbances under mesopic lighting conditions. In Stage 1 at Day 1 (Screening/Baseline), subjects will be randomized 1:1 to 1 of 2 treatment arms and stratified by iris color (light/dark irides): • Daily evening POS (n=100) • Daily evening placebo (ie, POS vehicle; n=100) The study eye for the primary endpoint will be the eye that meets Inclusion Criteria #6, #7, and #8 at Screening. In the case in which both eyes meet Inclusion Criteria #6, #7, and #8, the study eye will be the eye with the worse mLCVA. In the case in which both eyes have the same Screening mLCVA, the study eye will be the right eye. VA assessments will be made on the study eye, fellow eye, and binocularly. Study medication will be administered as a single drop in each eye by site staff on Day 1. Starting Day 2, study medication will be self-administered by the subject for approximately 6 weeks as a single drop in each eye in the evening near bedtime. On the evening prior to each office visit, subjects should dose as close as possible to 12 hours prior to the scheduled time for each office visit. All subjects will have safety and efficacy assessments at each office visit during Stage 1. In optional Stage 2 (after 6 weeks of treatment), subjects who

	consent to continue the study will continue to self-administer study medication as a single drop in each eye in the evening near bedtime on a daily basis for 42 additional weeks. All subjects will have safety assessments at each office visit during optional Stage 2.
Subject Population	Approximately 200 randomized subjects experiencing post−refractive surgery visual disturbances
Inclusion Criteria	1. Males or females ≥ 18 years of age 2. Previous history of refractive surgery (eg, PRK, LASIK, SMILE, and RK) and have subject-reported night vision disturbances (eg, glare, halos, and/or starbursts). Symptoms must have been first noted within 2 months following refractive surgery 3. Able to independently comply with all protocol-mandated procedures and to attend all scheduled office visits 4. Able and willing to give written consent to participate in this study 5. Able to self-administer study medication ***Inclusion criteria #6, #7, and #8 must all be met in the same eye:*** 6. PD ≥ 5 mm under mesopic conditions in at least 1 eye. This test may be repeated once, following an additional 5 min of dark adaptation to the mesopic light conditions if the initial results do not meet this criterion 7. mLCVA ≤ 30 Early Treatment Diabetic Retinopathy Study (ETDRS) letters (20/63 Snellen or worse) in at least 1 eye using the Precision Vision Illuminator Cabinet with 5% translucent contrast chart and a mesopic filter at 4 m 8. [redacted]
Exclusion Criteria	**Ophthalmic (in either eye):** 1. Prior unresolved dry eye diagnosis, taking prescription drops for dry eye, or taking artificial tear drops routinely for dry eye 2. Prior history of fluctuating vision

	3. Clinically significant ocular disease as deemed by the Investigator (eg, untreated visually significant cataract, glaucoma, corneal edema, uveitis, severe keratoconjunctivitis sicca, retina degeneration, loss of visual field due to glaucoma or stroke, branch retinal vein occlusion, retina flare) that might interfere with the study 4. History or presence of corneal endothelial dystrophy (eg, Fuchs' dystrophy or presence of guttae) 5. Known hypersensitivity to any topical alpha-adrenoceptor antagonists 6. Known allergy or contraindication to any component of the vehicle formulation 7. History of cauterization of the punctum or punctal plug (silicone or collagen) insertion or removal 8. Pseudophakic subjects with extended depth-of-focus or multifocal intraocular lenses (IOLs) 9. Ocular trauma, ocular surgery (eg, IOLs), or laser procedure (eg, LASIK, PRK, SMILE, and RK) within 6 months prior to Screening 10. Use of any topical prescription or over-the-counter (OTC) ophthalmic medications of any kind (including artificial tear drops) within 7 days prior to Screening until study completion, with the exception of lid scrubs with OTC products (eg, OCuSOFT® lid scrub, SteriLid®, baby shampoo, etc.) 11. Recent or current evidence of ocular infection or inflammation (such as current evidence of clinically significant blepharitis, conjunctivitis, or a history of herpes simplex or herpes zoster keratitis at Screening). Subjects must be symptom free for at least 7 days prior to Screening 12. History of diabetic retinopathy, diabetic macular edema, or dry or wet macular degeneration 13. History of any traumatic (surgical or nonsurgical) or nontraumatic condition affecting the pupil or iris (eg, irregularly shaped pupil, neurogenic pupil disorder, iris atrophy, iridotomy, iridectomy, etc.) 14. Unwilling or unable to discontinue use of contact lenses at least 1 hour prior to Screening for soft contact lenses or at least 8 hours prior to Screening for hard gas-permeable contact lenses, and at least 8 hours (for both types of lenses) prior to all other office visits

	15. Previously undiagnosed dry eye, at the determination of the Investigator. Dry eye diagnosis should be based on one of the following dry eye test results: tear break-up time < 5 seconds, or corneal fluorescein staining ≥ Grade 2 in the inferior zone or ≥ Grade 1 in the central zone using the National Eye Institute scale **Systemic:** 16. Known hypersensitivity or contraindication to alpha- and/or beta-adrenoceptor antagonists (eg, chronic obstructive pulmonary disease or bronchial asthma; abnormally low BP or HR; second- or third-degree heart blockage or congestive heart failure; or severe diabetes as defined below) a. Predisposition to severe hypoglycemia (2 or more serious hypoglycemic episodes requiring assistance within 12 months prior to Screening) b. Any hospitalization or emergency room visit due to poor diabetic control within 6 months prior to Screening c. Currently untreated diabetes mellitus or previously untreated subjects who initiated oral anti-diabetic medication or insulin within 3 months prior to Screening d. Any sign of diabetic retinopathy in either eye 17. Clinically significant systemic disease (eg, severe diabetes as previously defined, myasthenia gravis, cancer, hepatic, renal, endocrine, or cardiovascular disorders) that might interfere with the study 18. Initiation of treatment with or any changes to the current dosage, drug, or regimen of any systemic adrenergic or cholinergic drugs within 7 days prior to Screening or during the study 19. Participation in any investigational study within 30 days prior to Screening or during the study 20. Females of childbearing potential who are pregnant, nursing, planning a pregnancy during the study, or not using a medically acceptable form of birth control. Acceptable methods include the use of at least one of the following: intrauterine device, hormonal (oral, injection, patch, implant, ring), barrier with spermicide (condom, diaphragm), or abstinence. A female is considered to be of childbearing potential unless she is 1 year postmenopausal or 3 months post−surgical sterilization.

	All females of childbearing potential including those post−tubal ligation must have a negative urine pregnancy test result at each visit 21. Resting HR outside 50 to 110 beats per min at Screening. HR may be repeated **only once** if outside the specified range, following at least a 5-min rest period in the sitting position 22. Hypertension with resting diastolic BP > 105 mmHg or systolic BP > 160 mmHg at Screening. BP may be repeated **only once** if outside the specified range, following at least a 5-min rest period in the sitting position
Screening/Baseline Visit	Potential subjects are identified by the study center to schedule the Screening/Baseline Visit. The start of Screening includes an explanation of the study and signing of the informed consent form, a medical and ophthalmic history, demographics, HR and BP, urine pregnancy test (for females of childbearing potential only), and eligibility assessments. Prior to the eligibility assessments, the subject must be asked to complete the subject questionnaire. Then subjects will be assessed in each eye for PD and for mLCVA before and during illumination of the contralateral eye with a BAT system on the low setting (Inclusion Criteria #6–8). Subjects must meet all inclusion criteria (and the study eye of the subject must meet Inclusion Criteria #6, #7, and #8) and none of the exclusion criteria to be eligible for enrollment. Before further screening criteria are evaluated, the following assessments will be performed to establish baseline values: • Mesopic high-contrast visual acuity (mHCVA) • Slit lamp biomicroscopy Final screening must include a baseline intraocular pressure (IOP) measurement (using a Tono-Pen or Goldmann applanation tonometry) and ophthalmoscopy (non-dilated). For subjects passing all eligibility criteria, the Screening Visit becomes the Baseline Visit. The subject will then be randomized into the study. The first dose of study medication will be administered at the study site by site staff at the Baseline Visit/Day 1 and mLCVA assessments will be made at 0.5, 1, and 3 hours post-dose. The subject diary for Stage 1 will be dispensed. Note that all Visit 1 (Screening/Baseline) assessments are

	recommended to be performed on the same day; however, in instances in which subjects are unable to complete the full day of assessments for Visit 1, subjects may be scheduled to return for the completion of Visit 1 within 1 week of Screening. If Visit 1 is split over 2 separate days, all eligibility (Screening) assessments up to and including randomization must occur on the first day, and then the subject should return to complete all post-randomization (Baseline) assessments starting with treatment dosing on the second day of the visit.
Treatment Visits	There will be 5 office visits in Stage 1: Day 1 (Screening/ Baseline)/Visit 1, Day 3/Visit 2, Day 8/Visit 3, Day 15/Visit 4 (primary endpoint), and Week 6 (End of Stage 1)/Visit 5; mLCVA evaluations will be performed on each of these days. Other assessments will be performed as defined in the Schedule of Visits and Procedures. Following completion of the 6-week treatment period, subjects will have the option of continuing study medication for an additional 42 weeks (optional Stage 2), with 7 office visits at 12, 18, 24, 30, 36, 42, and 48 weeks.
Number of Investigational Sites	Approximately 40 sites in the United States
Estimated Total Sample Size	Approximately 200 randomized subjects
Sample Size Justification	A total of approximately 200 subjects will be enrolled and randomized into the study in a 1:1 ratio to 1 of the 2 treatment arms (POS or placebo), with a stratification by light/dark irides. The primary efficacy endpoint will be met if subjects show a positive effect for POS ($\alpha = 0.05$ significance, 2-sided) at Day 15 in the study eye. A total of 200 subjects will provide 89% power to detect a difference of 16% between the POS and placebo groups in percent of subjects with $\geq$ 15 ETDRS letters improvement in monocular mLCVA at Day 15 compared to Baseline. This calculation is based on a 5% placebo effect and a 21% POS treatment effect. To test for tachyphylaxis, a sample size of 100 subjects receiving POS provides greater than 90% power ($\alpha = 0.05$ significance) to detect a 5-letter or more inferiority of the change in monocular mLCVA after 6 weeks of daily dosing

	compared to the best change in mLCVA during the first month of the study, assuming a standard deviation of 9 letters.
Primary Efficacy Endpoint	Percent of subjects with ≥ 15 ETDRS letters (≥ 3 lines) improvement in the study eye in mLCVA compared to Baseline (Day 1 pre-dose) at Day 15
Secondary and Exploratory Efficacy Endpoints	Secondary efficacy endpoints will include the following: • Percent of subjects with ≥ 10 and ≥ 15 ETDRS letters improvement in mLCVA compared to Baseline (Day 1 pre-dose) at Days 3, 8, and 15 (≥10 ETDRS letters only) and Week 6 • Mean and change from Baseline (Day 1 pre-dose) in mLCVA at 0.5, 1, and 3 hours post-dose on Day 1 • Percent of subjects with ≥ 10 and ≥ 15 ETDRS letters improvement in mHCVA compared to Baseline (Day 1 pre-dose) at Days 3, 8, and 15 and Week 6 • Mean and change from Baseline (Day 1 pre-dose) in mLCVA and mHCVA at Days 3, 8, and 15 and Week 6 • Mean and change from Baseline (Day 1 pre-dose) in PD at Days 3, 8, and 15 and Week 6 • Change in subject questionnaire responses compared to Baseline (Day 1 pre-dose) at Days 3, 8, and 15 and Week 6 Exploratory efficacy endpoints include all the secondary VA and PD efficacy endpoints, with the analysis of VA data performed on the fellow eye and binocular test results, and the analysis of PD data performed on the fellow eye. Measurements: • [redacted]

Safety Endpoints	Secondary safety and tolerability endpoints measured at all office visits (refer to the Schedule of Visits and Procedures for specifics) will include the following: • Adverse events • IOP • Percentage of subjects with ≥ 5, ≥ 10, and ≥ 15 letters loss in best-corrected distance visual acuity (BCDVA) from Baseline (Day 1 pre-dose) at 12 hours post-dose • Change from Baseline (Day 1 pre-dose) in BCDVA at 12 hours post-dose • Biomicroscopy • Ophthalmoscopy • Vital signs (HR and BP) Measurements: • [redacted] [redacted]
Study Medications, Dose and Mode of Administration	0.75% Phentolamine Ophthalmic Solution (POS): For those subjects randomized to POS treatment, 1 drop of POS will be self-administered by the subject in each eye once daily (QD) in the evening near bedtime for approximately 6 weeks during Stage 1. Placebo (POS vehicle): For those subjects randomized to placebo treatment, 1 drop of POS vehicle will be self-administered by the subject in each eye QD in the evening near bedtime for approximately 6 weeks during Stage 1. Study medication doses taken the evening prior to an in-office visit should be administered as close as possible to 12 hours prior to the scheduled time for that office visit. During optional Stage 2, the subject will continue to self-administer study medication in each eye QD in the evening near bedtime for an additional 42 weeks.

Duration of Subject Participation and Study	The length of subject participation is approximately 6 weeks for Stage 1, with 5 clinic visits summarized below: • Visit 1 (Screening/Baseline)/Day 1 • Visit 2 (Stage 1 Treatment)/Day 3 • Visit 3 (Stage 1 Treatment)/Day 8 (± 1 day) • Visit 4 (Stage 1 Treatment)/Day 15 (± 1 day) • Visit 5 (End of Stage 1)/Week 6 (± 3 days) At Visit 5, subjects agreeing to continue to Stage 2 will be reconsented. The length of optional Stage 2 will continue for approximately an additional 42 weeks, with 7 clinic visits summarized below: • Visit 6 (Stage 2 Follow-Up [FU])/Week 12 (± 3 days) • Visit 7 (Stage 2 FU)/Week 18 (± 3 days) • Visit 8 (Stage 2 FU)/Week 24 (± 3 days) • Visit 9 (Stage 2 FU)/Week 30 (± 3 days) • Visit 10 (Stage 2 FU)/Week 36 (± 3 days) • Visit 11 (Stage 2 FU)/Week 42 (± 3 days) • Visit 12 (End of Study)/Week 48 (± 3 days)

TABLE OF CONTENTS

INVESTIGATOR'S AGREEMENT 3
PROCEDURES IN CASE OF EMERGENCY 4
1 STUDY SUMMARY 5
TABLE OF CONTENTS 15
LIST OF TABLES 17
2 ABBREVIATIONS AND TERMS 18
3 INTRODUCTION 20
3.1 Investigational Products 20
3.2 Findings From Nonclinical and Clinical Studies 20
3.3 Design Justification 20
4 OBJECTIVES AND PURPOSE 23
5 STUDY DESIGN 24
5.1 Primary Endpoint 24
5.2 Secondary Endpoints 24
5.3 Exploratory Endpoints 25
5.4 Description and Schedule of Visits and Procedures 26
5.4.1 Visit 1 (Screening/Baseline)/Day 1 29
5.4.2 Visit 2 (Stage 1 Treatment)/Day 3 29
5.4.3 Visit 3 (Stage 1 Treatment)/Day 8 ± 1 day 30
5.4.4 Visit 4 (Stage 1 Treatment)/Day 15 ± 1 day 31
5.4.5 Visit 5 (End of Stage 1)/Week 6 ± 3 days 31
5.4.6 Visit 6 (Stage 2 Follow-Up)/Week 12 ± 3 days 32
5.4.7 Visit 7 (Stage 2 Follow-Up)/Week 18 ± 3 days 32
5.4.8 Visit 8 (Stage 2 Follow-Up)/Week 24 ± 3 days 33
5.4.9 Visit 9 (Stage 2 Follow-Up)/Week 30 ± 3 days 33
5.4.10 Visit 10 (Stage 2 Follow-Up)/Week 36 ± 3 days 33
5.4.11 Visit 11 (Stage 2 Follow-Up)/Week 42 ± 3 days 34
5.4.12 Visit 12 (End of Study)/Week 48 ± 3 days 34
5.4.13 Unscheduled Visits 35
5.4.14 Visit Variation 35
5.5 Measures Taken to Minimize/Avoid Bias 35
5.6 Study Medications 35
5.6.1 Packaging and Labeling 36
5.6.2 Storage of Study Medication and Dispensing 36
5.6.3 Study Medication Administration 36

5.6.4 Study Medication Accountability 37
5.7 Expected Duration of Subject Participation 37
5.8 Randomization and Procedure for Breaking the Code 38
5.9 Collection of Data 38
5.10 Completed Subject 39
5.11 Non-completing Subject 39
5.11.1 Study Medication Discontinuation 39
5.11.2 Reasons for Withdrawal From Study 39
5.11.3 Entire Study Terminated 39
5.11.4 Actions After Discontinuation 40
5.12 Completed Study 40
6 SUBJECT INCLUSION AND EXCLUSION CRITERIA 41
6.1 Subject Inclusion Criteria 41
6.2 Subject Exclusion Criteria 41
7 TREATMENT OF SUBJECTS 44
7.1 Treatment Adherence 44
7.2 Concomitant Medications 44
8 ASSESSMENT OF EFFICACY 46
8.1 Specification of the Efficacy Parameters 46
8.2 Assessing, Recording, and Analyzing Efficacy Parameters 46
9 ASSESSMENT OF SAFETY 47
9.1 Specification of Safety Parameters 47
9.2 Adverse Events and Serious Adverse Events 47
9.2.1 Adverse Event Definitions 47
9.2.2 Follow-Up of Subjects After Adverse Events 51
10 STATISTICS 52
10.1 Sample Size 52
10.2 Analysis Populations 52
10.3 Statistical Methods 52
10.3.1 General Considerations 52
10.3.2 Demographic and Baseline Characteristics 53
10.3.3 Subject Disposition 53
10.3.4 Medical History and Prior/Concomitant Medications 53
10.3.5 Estimands for Efficacy 53
10.3.6 Analysis of Efficacy 54
10.3.7 Analysis of Safety 55
10.4 Interim Analyses 56
10.5 Final Analysis 56

10.6 Procedure for Accounting for Missing, Unused, or Spurious Data 56
10.7 Procedure for Reporting Deviations From the Statistical Plan 56
11 DIRECT ACCESS TO SOURCE DATA AND DOCUMENTS 57
12 QUALITY CONTROL AND QUALITY ASSURANCE.....58
13 ETHICAL CONSIDERATIONS AND GOOD CLINICAL PRACTICE COMPLIANCE.....59
13.1 Good Clinical Practice Compliance 59
13.2 Institutional Review Board 59
13.3 Protocol Deviations/Violations 59
13.4 Informed Consent Requirements 59
14 DATA HANDLING AND RECORD KEEPING 61
14.1 Data Entry 61
14.2 Data Quality Control and Reporting 61
14.3 Archiving of Data 61
14.4 Records Retention 61
14.5 Amendments to the Protocol.....61
15 BIBLIOGRAPHY.....62
16 SUMMARY OF CHANGES 64
APPENDIX 1: IRIS COLOR CHART 65
APPENDIX 2: SUBJECT QUESTIONNAIRE.....66
APPENDIX 3: ADRENERGIC AND CHOLINERGIC DRUGS.....70

LIST OF TABLES

Table 1 Stage 1 Schedule of Visits and Procedures 27
Table 2 Optional Stage 2 Schedule of Visits and Procedures 28
Table 3 Phentolamine Mesylate Drug Substance and Drug Product Identifiers 35

2 ABBREVIATIONS AND TERMS

Abbreviation	Full term
°C	degrees Celsius
°F	degree Fahrenheit
AE	adverse event
ANCOVA	analysis of covariance
ARP	All Randomized Population
BAT	Brightness Acuity Tester
BCDVA	best-corrected distance visual acuity
BP	blood pressure
bpm	beats per min
CI	confidence interval
COVID-19	Coronavirus Disease 2019
CRA	Clinical Research Associate
CRO	contract research organization
D	diopter
DCNVA	distance-corrected near visual acuity
DLD	dim light visual disturbance(s)
eCRF	electronic Case Report Form
ETDRS	Early Treatment Diabetic Retinopathy Study
FDA	Food and Drug Administration
FU	follow-up
GCP	Good Clinical Practice
HR	heart rate
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation
IOL	intraocular lens

IOP	intraocular pressure
IRB	Institutional Review Board
ITT	Intention-to-Treat
LASIK	laser-assisted in situ keratomileusis
LS	least-squares
MedDRA	Medical Dictionary for Regulatory Activities
mHCVA	mesopic high-contrast visual acuity (BCDVA)
mITT	Modified Intention-to-Treat
mLCVA	mesopic low-contrast visual acuity (BCDVA)
NA	not applicable
OTC	over-the-counter
OU	oculus uterque (both eyes)
PD	pupil diameter
pLCVA	photopic low-contrast visual acuity (BCDVA)
POS	0.75% Phentolamine Ophthalmic Solution or 1% Phentolamine Mesylate Ophthalmic Solution
PP	Per Protocol
PRK	photorefractive keratectomy
QD	once daily
RK	radial keratotomy
SAE	serious adverse event
SAP	Statistical Analysis Plan
SMILE	small-incision lenticule extraction
SOC	system organ class
TEAE	treatment-emergent adverse event
US	United States
USP	United States Pharmacopeia
VA	visual acuity
VND-Q	Vision and Night Driving Questionnaire

3 INTRODUCTION

3.1 Investigational Products

The test product is 0.75% Phentolamine Ophthalmic Solution (POS Eye Drops), a non-selective alpha-1 and alpha-2 adrenergic antagonist. Note that the concentration of 0.75% refers to phentolamine free base and is the same as and used in place of 1% phentolamine mesylate drug substance, which was how POS had been described in all prior studies. Placebo control is POS vehicle alone.

3.2 Findings From Nonclinical and Clinical Studies

Detailed findings from nonclinical and clinical studies for phentolamine mesylate or phentolamine ophthalmic solutions and potential risk are provided in the Investigator's Brochure (IB; 2022).

POS has been studied in a total of 12 clinical trials (three Phase 1, five Phase 2, and four Phase 3) enrolling over 1,100 subjects (including subjects 3 years of age and up, with over 650 subjects treated with POS) and has demonstrated promising clinical data for use in multiple ophthalmic indications.

In prior clinical trials, POS has demonstrated a consistent ability to decrease pupil diameter (PD) by approximately 20% to 30% (~1–1.5 mm) in both mesopic and photopic conditions (McDonald 2010; Holladay 2018; Pepose 2020). POS was found to be efficacious for over 24 hours, with an onset of action of approximately 30 to 60 min. POS was well tolerated at single doses and multiple doses up to 14 days. Safety of the subjects in these trials was evaluated by adverse event (AE) monitoring, physical examinations, and vital sign assessments. Across all trials, no serious adverse events (SAEs) have been reported. No deaths occurred in any of the trials. No clinically meaningful changes were observed in physical examinations or vital signs, including blood pressure (BP) and heart rate (HR). Adverse events reported were mild to moderate in intensity, with the most common being transient conjunctival hyperemia (eye redness) and ocular irritation; however, POS dosing near bedtime was observed to mitigate or minimize these side effects during the daytime.

3.3 Design Justification

Low-light visual disturbance following refractive surgery affects patients under mesopic or scotopic conditions. Night vision disturbance involves photic phenomena, such as glare, halos, and starbursts, which can be a result of a combination of ocular aberrations and light scatter. These visual disturbances can be debilitating to many everyday activities, especially driving. The light emitted by traffic lights and other cars scatters and obscures most of the visual field, making driving in dim light conditions hazardous. Glare can be particularly dangerous while driving. Up to 3% of individuals with refractive procedures are unable to function visually at night (Pop 2004; Fan-Paul 2002; Holladay 1999; Monestam 2006).

Night vision and dim light visual disturbances can be mitigated by miosis, in which the smaller pupil blocks the aberrant peripheral rays of light, selectively allowing passage of the more centrally focused rays (Martinez 1998). A miotic change in the pupil size can be achieved by modulating 1 of 2 or both opposing sets of muscles – the iris sphincter

muscles controlled by the cholinergic nervous system and the iris dilator muscles controlled by the adrenergic nervous system (Yoshitomi 1985; Steinhauer 2004). An alpha-1 antagonist approach will act on the iris dilator muscle and produce a miotic effect (Yu 2002). It is proposed that a moderate pharmacologic reduction of pupil size (20–25%) by the application of an alpha-1 antagonist has the potential to mitigate night vision disturbance following refractive surgery in many affected individuals.

The biggest challenge for the treatment of visual disturbance under low-light conditions in post−refractive surgery patients is the lack of safe, tolerable, convenient, and effective treatment options. There is no United States (US) Food and Drug Administration (FDA)-approved treatment for night vision disturbance in these patients. Some commonly used tools, such as tinted glasses, are not effective and in fact may worsen patients' vision at night. Off-label use of approved miotic agents, such as regular-strength pilocarpine, is unsuitable for the treatment of these patients because these agents reduce pupil size to a degree that may impede safe night vision, may cause loss of accommodation, and increase the risk of retinal detachment (Isopto prescribing information; Vuity prescribing information).

Phentolamine is a non-selective alpha-1 and alpha-2 adrenergic antagonist acting on adrenergic receptors and is known to inhibit contraction of the iris dilator muscle, resulting in a smaller pupil size.

Efficacy Results From the LYNX-1 Study in Subjects With Dim Light Visual Disturbance

LYNX-1 (OPI-NYXDLD-301) was a Phase 3, randomized, double-masked, placebo-controlled, multicenter study throughout the US of POS compared with placebo ophthalmic solution in subjects with dim light visual disturbance (DLD). In this trial, 145 subjects who experienced vision impairment under dim light conditions were randomized to receive either POS or placebo. Each subject was randomized 1:1 to treatment with POS or placebo and stratified by iris color (light/dark irides). Treatment was self-administered in each eye once daily (QD) near bedtime for 14 days. Efficacy was measured by assessment of mesopic low-contrast visual acuity (mLCVA), photopic low-contrast visual acuity (pLCVA), best-corrected distance visual acuity (BCDVA), mesopic high-contrast visual acuity (mHCVA), distance-corrected near visual acuity (DCNVA), mesopic high-contrast DCNVA, mesopic PD, photopic PD, subject questionnaire, and wavefront aberrometry. Safety assessments included conjunctival hyperemia, slit lamp biomicroscopy, ophthalmoscopy, intraocular pressure (IOP), physical exams, and HR and BP.

[redacted]

[redacted]

4 OBJECTIVES AND PURPOSE

The LYNX-2 study is a randomized, placebo-controlled, double-masked, multiple-dose study of the safety and efficacy of POS in subjects with post−refractive surgery visual disturbances.

The primary objectives of this study are:

- To evaluate the efficacy of POS to improve mLCVA in subjects with post−refractive surgery visual disturbances
- To evaluate the efficacy of POS to improve VA under various lighting conditions in subjects with post−refractive surgery visual disturbances
- To evaluate tachyphylaxis of the therapeutic response to POS in subjects with post−refractive surgery visual disturbances
- To evaluate the safety of POS in subjects with post−refractive surgery visual disturbances

5 STUDY DESIGN

This is a randomized, placebo-controlled, double-masked, 2-stage, multiple-dose, multicenter, Phase 3 study in approximately 200 subjects with post−refractive surgery visual disturbances under mesopic lighting conditions.

In Stage 1 at Day 1 (Screening/Baseline), subjects will be randomized 1:1 to 1 of 2 treatment arms and stratified by iris color (light/dark irides; Appendix 1):

- Daily evening POS (n=100)
- Daily evening placebo (ie, POS vehicle; n=100)

The study eye for the primary endpoint will be the eye that meets Inclusion Criteria #6, #7, and #8 at Screening. In the case in which both eyes meet Inclusion Criteria #6, #7, and #8, the study eye will be the eye with the worse mLCVA. In the case in which both eyes have the same Screening mLCVA, the study eye will be the right eye. Visual acuity assessments will be made on the study eye, fellow eye, and binocularly.

Study medication will be administered as a single drop in each eye by site staff on Day 1. Starting Day 2, study medication will be self-administered by the subject for approximately 6 weeks as a single drop in each eye in the evening near bedtime. On the evening prior to each office visit, subjects should dose as close as possible to 12 hours prior to the scheduled time for each office visit. All subjects will have safety and efficacy assessments at each office visit during Stage 1.

In optional Stage 2 (after 6 weeks of treatment), subjects who consent to continue the study will continue to self-administer study medication as a single drop in each eye in the evening near bedtime on a daily basis for 42 additional weeks. All subjects will have safety assessments at each office visit during optional Stage 2.

5.1 Primary Endpoint

The primary efficacy endpoint is the percent of subjects with ≥ 15 Early Treatment Diabetic Retinopathy Study (ETDRS) letters (≥ 3 lines) improvement in the study eye in mLCVA compared to Baseline (Day 1 pre-dose) at Day 15.

5.2 Secondary Endpoints

Secondary efficacy endpoints will include the following:

- Percent of subjects with ≥ 10 and ≥ 15 ETDRS letters improvement in mLCVA compared to Baseline (Day 1 pre-dose) at Days 3, 8, and 15 (≥ 10 ETDRS letters only) and Week 6
- Mean and change from Baseline (Day 1 pre-dose) in mLCVA at 0.5, 1, and 3 hours post-dose on Day 1
- Percent of subjects with ≥ 10 and ≥ 15 ETDRS letters improvement in mHCVA compared to Baseline (Day 1 pre-dose) at Days 3, 8, and 15 and Week 6
- Mean and change from Baseline (Day 1 pre-dose) in mLCVA and mHCVA at Days 3, 8, and 15 and Week 6

- Mean and change from Baseline (Day 1 pre-dose) in PD at Days 3, 8, and 15 and Week 6
- Change in subject questionnaire responses compared to Baseline (Day 1 pre-dose) at Days 3, 8, and 15 and Week 6

Efficacy measurements:

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

Secondary safety and tolerability endpoints measured at all office visits (Table 1 and Table 2) will include the following:

- Adverse events
- Intraocular pressure
- Percentage of subjects with ≥ 5, ≥ 10, and ≥ 15 letters loss in BCDVA from Baseline (Day 1 pre-dose) at 12 hours post-dose
- Change from Baseline (Day 1 pre-dose) in BCDVA at 12 hours post-dose
- Biomicroscopy
- Corneal specular microscopy, if available
- Ophthalmoscopy
- Vital signs (HR and BP)

Safety measurements:

- [redacted]
- [redacted]

5.3 Exploratory Endpoints

Exploratory efficacy endpoints include all the secondary VA and PD efficacy endpoints, with the analysis of VA data performed on the fellow eye and binocular test results, and the analysis of PD data performed on the fellow eye.

5.4 Description and Schedule of Visits and Procedures

Approximately 200 subjects ≥ 18 years of age will be randomized in a 1:1 ratio to 1 of 2 treatment arms (POS or placebo). Randomization will be stratified by iris color (light/dark irides). Study procedures are shown in detail in Table 1 for Stage 1 and in Table 2 for optional Stage 2.

Table 1 Stage 1 Schedule of Visits and Procedures

Visit number	1 [a]				2	3	4	5
Visit timing (window)	Screening/Baseline Day 1				Day 3	Day 8 (± 1 day)	Day 15 (± 1 day)	End of Stage 1 Week 6 (± 3 days)
Hours (window)	0	0.5 (± 5 min)	1 (± 5 min)	3 (± 10 min)	NA	NA	NA	NA
Informed consent	X							X
Medical/Ophthalmic history	X							
Demographics	X							
Prior/Concomitant medications [b]	X				X	X	X	X
Urine pregnancy test [c]	X				X	X	X	X
HR/BP [d]	X				X	X	X	X
Manifest refraction	X							
Subject questionnaire [e]	X				X	X	X	X
BCDVA [f]	X				X	X	X	X
PD [g]	X				X	X	X	X
mLCVA [f]	X	X	X	X	X	X	X	X
[redacted]	X							
mHCVA [f]	X				X	X	X	X
Slit lamp biomicroscopy	X				X	X	X	X
Automated corneal specular microscopy, if available	X				X	X	X	X
Dry eye test [h]	X							
IOP [i]	X				X	X	X	X
Ophthalmoscopy [j]	X				X	X	X	X
Adverse events	X				X	X	X	X
Treatment dosing [k]	X							
Treatment dispensed/collected	X [a]							X
Subject diary dispensed/reviewed [l]	X				X	X	X	X
Study medication reminder [m]	X				X	X	X	

[redacted]BCDVA, best-corrected distance visual acuity; BP, blood pressure; D, diopter; HR, heart rate; IOP, intraocular pressure; mHCVA, mesopic high-contrast visual acuity (BCDVA); mLCVA, mesopic low-contrast visual acuity (BCDVA); NA, not applicable; PD, pupil diameter; VA, visual acuity.

[a] If the subject meets all the inclusion criteria and none of the exclusion criteria, this Screening Visit becomes the Baseline Visit. A randomization number is assigned after the subject is qualified.

[b] This includes asking if subjects have been diagnosed with dry eye, take prescription drops for dry eye, or take artificial tear drops routinely for dry eye. Investigators will note changes to concomitant medications at any time throughout the study.

[c] Urine pregnancy test is for females of childbearing potential only.

[d] HR and BP can be measured manually or with available digital devices.
[e] Subject questionnaire will be a brief symptom survey.
[f] VA assessments will be conducted first in each eye individually, followed by binocular assessment at Screening; thereafter, study eye will be assessed first, followed by fellow eye and then binocular.
[redacted]
[h] Dry eye test will include both tear break-up test (performed first) and corneal fluorescein staining test.
[redacted]
[j] Direct or indirect ophthalmoscopy will be performed without dilation. Use of 90 D lens (indirect) is allowed.
[k] Treatment dosing will occur in the office at Visit 1.
[l] Subject diary will be provided to subjects at Visit 1, and a new subject diary will be provided to subjects consenting to participate in optional Stage 2 at Visit 5.
[m] All subjects will be reminded to instill treatment approximately 12 hours prior to the scheduled time of their next office visit.

Table 2 Optional Stage 2 Schedule of Visits and Procedures

Visit number	6	7	8	9	10	11	12
Visit timing (window)	Week 12 (± 3 days)	Week 18 (± 3 days)	Week 24 (± 3 days)	Week 30 (± 3 days)	Week 36 (± 3 days)	Week 42 (± 3 days)	End of Study Week 48 (± 3 days)
Prior/Concomitant medications [a]	X	X	X	X	X	X	X
Urine pregnancy test [b]	X	X	X	X	X	X	X
HR/BP [c]	X	X	X	X	X	X	X
Subject questionnaire [d]	X	X	X	X	X	X	X
BCDVA [e]	X	X	X	X	X	X	X
Slit lamp biomicroscopy	X	X	X	X	X	X	X
Automated corneal specular microscopy, if available	X	X	X	X	X	X	X
IOP [f]	X	X	X	X	X	X	X
Ophthalmoscopy [g]	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X
Treatment dispensed/collected	X	X	X	X	X	X	X
Subject diary reviewed	X	X	X	X	X	X	X

BCDVA, best-corrected distance visual acuity; BP, blood pressure; D, diopter; HR, heart rate; IOP, intraocular pressure.
[a] This includes asking if subjects have been diagnosed with dry eye, take prescription drops for dry eye, or take artificial tear drops routinely for dry eye. Investigators will note changes to concomitant medications at any time throughout the study.
[b] Urine pregnancy test is for females of childbearing potential only.
[c] HR and BP can be measured manually or with available digital devices.
[d] Subject questionnaire will be a brief symptom survey.
[e] Study eye BCDVA will be assessed first, followed by fellow eye and then binocular.
[redacted]
[g] Direct or indirect ophthalmoscopy will be performed without dilation. Use of 90 D lens (indirect) is allowed.

5.4.1 Visit 1 (Screening/Baseline)/Day 1

Potential subjects are identified by the study center to schedule the Screening/Baseline Visit.

The start of Screening includes an explanation of the study and signing of the informed consent form (ICF), a medical and ophthalmic history, demographics, HR and BP, urine pregnancy test (for females of childbearing potential only), and eligibility assessments.

Prior to the eligibility assessments, the subject must be asked to complete the subject questionnaire. Then subjects will be assessed in each eye for PD and for mLCVA [redacted] Inclusion Criteria #6–8).

Subjects must meet all inclusion criteria (and the study eye of the subject must meet Inclusion Criteria #6, #7, and #8) and must not meet any ophthalmic Exclusion Criteria to be eligible for enrollment.

Before further screening criteria are evaluated, the following assessments will be performed to establish baseline values:

- Mesopic high-contrast VA
- Slit lamp biomicroscopy

Final screening must include a baseline IOP measurement (using a Tono-Pen or Goldmann applanation tonometry) and ophthalmoscopy (non-dilated).

For subjects passing all eligibility criteria, the Screening Visit becomes the Baseline Visit. The subject will then be randomized into the study. The first dose of study medication will be administered at the study site by site staff at the Baseline Visit/Day 1 and mLCVA assessments will be made at 0.5, 1, and 3 hours post-dose. The subject diary for Stage 1 will be dispensed.

Note that all Visit 1 (Screening/Baseline) assessments are recommended to be performed on the same day; however, in instances in which subjects are unable to complete the full day of assessments for Visit 1, subjects may be scheduled to return for the completion of Visit 1 within 1 week of Screening. If Visit 1 is split over 2 separate days, all eligibility (Screening) assessments up to and including randomization must occur on the first day, and then the subject should return to complete all post-randomization (Baseline) assessments starting with treatment dosing on the second day of the visit.

The first dose of at-home study medication will be administered by subjects as 1 drop in both eyes (OU) on Day 2 as close as possible to 12 hours prior to the scheduled time for Visit 2/Day 3.

5.4.2 Visit 2 (Stage 1 Treatment)/Day 3

During the Day 3 Visit, the following safety and efficacy measurements will be made approximately 12 hours after the dose of study medication (Time 0 min):

Concomitant medications

Urine pregnancy test (for females of childbearing potential only)

Heart rate and BP

Subject questionnaire

Pupil diameter (mesopic)

Study eye photopic BCDVA (followed by fellow eye and then binocular BCDVA)

Study eye mLCVA (followed by fellow eye and then binocular mLCVA)

Study eye mHCVA (followed by fellow eye and then binocular mHCVA)

Slit lamp biomicroscopy

Corneal specular microscopy, if available

Intraocular pressure

Ophthalmoscopy

Adverse events

Subject diary review

Study medication reminder

5.4.3 Visit 3 (Stage 1 Treatment)/Day 8 ± 1 day

During the Day 8 Visit, the following safety and efficacy measurements will be made approximately 12 hours after the dose of study medication (Time 0 min):

Concomitant medications

Urine pregnancy test (for females of childbearing potential only)

Heart rate and BP

Subject questionnaire

Pupil diameter (mesopic)

Study eye photopic BCDVA (followed by fellow eye and then binocular BCDVA)

Study eye mLCVA (followed by fellow eye and then binocular mLCVA)

Study eye mHCVA (followed by fellow eye and then binocular mHCVA)

Slit lamp biomicroscopy

Corneal specular microscopy, if available

Intraocular pressure

Ophthalmoscopy

Adverse events

Subject diary review

Study medication reminder

5.4.4 Visit 4 (Stage 1 Treatment)/Day 15 ± 1 day

During the Day 15 Visit, the following safety and efficacy measurements will be made approximately 12 hours after the dose of study medication (Time 0 min):

Concomitant medications

Urine pregnancy test (for females of childbearing potential only)

Heart rate and BP

Subject questionnaire

Pupil diameter (mesopic)

Study eye photopic BCDVA (followed by fellow eye and then binocular BCDVA)

Study eye mLCVA (followed by fellow eye and then binocular mLCVA)

Study eye mHCVA (followed by fellow eye and then binocular mHCVA)

Slit lamp biomicroscopy

Corneal specular microscopy, if available

Intraocular pressure

Ophthalmoscopy

Adverse events

Subject diary review

Study medication reminder

5.4.5 Visit 5 (End of Stage 1)/Week 6 ± 3 days

During the Week 6 Visit, the following safety and efficacy measurements will be made approximately 12 hours after the dose of study medication (Time 0 min):

Concomitant medications

Urine pregnancy test (for females of childbearing potential only)

Heart rate and BP

Subject questionnaire

Pupil diameter (mesopic)

Study eye photopic BCDVA (followed by fellow eye and then binocular BCDVA)

Study eye mLCVA (followed by fellow eye and then binocular mLCVA)

Study eye mHCVA (followed by fellow eye and then binocular mHCVA)

Slit lamp biomicroscopy

Corneal specular microscopy, if available

Intraocular pressure

Ophthalmoscopy

Adverse events

Informed consent for Stage 2 (optional)

Study medication collected from Stage 1 and new study medication dispensed for optional Stage 2

Subject diary collected from Stage 1 and new diary dispensed for optional Stage 2

5.4.6 Visit 6 (Stage 2 Follow-Up)/Week 12 ± 3 days

During the Week 12 Visit, the following safety measurements will be made:

Concomitant medications

Urine pregnancy test (for females of childbearing potential only)

Heart rate and BP

Subject questionnaire

Study eye photopic BCDVA (followed by fellow eye and then binocular BCDVA)

Slit lamp biomicroscopy

Corneal specular microscopy, if available

Intraocular pressure

Ophthalmoscopy

Adverse events

Study medication collected and new study medication dispensed

Subject diary review

5.4.7 Visit 7 (Stage 2 Follow-Up)/Week 18 ± 3 days

During the Week 18 Visit, the following safety measurements will be made:

Concomitant medications

Urine pregnancy test (for females of childbearing potential only)

Heart rate and BP

Subject questionnaire

Study eye photopic BCDVA (followed by fellow eye and then binocular BCDVA)

Slit lamp biomicroscopy

Corneal specular microscopy, if available

Intraocular pressure

Ophthalmoscopy

Adverse events

Study medication collected and new study medication dispensed

Subject diary review

5.4.8 Visit 8 (Stage 2 Follow-Up)/Week 24 ± 3 days

During the Week 24 Visit, the following safety measurements will be made:

Concomitant medications

Urine pregnancy test (for females of childbearing potential only)

Heart rate and BP

Subject questionnaire

Study eye photopic BCDVA (followed by fellow eye and then binocular BCDVA)

Slit lamp biomicroscopy

Corneal specular microscopy, if available

Intraocular pressure

Ophthalmoscopy

Adverse events

Study medication collected and new study medication dispensed

Subject diary review

5.4.9 Visit 9 (Stage 2 Follow-Up)/Week 30 ± 3 days

During the Week 30 Visit, the following safety measurements will be made:

Concomitant medications

Urine pregnancy test (for females of childbearing potential only)

Heart rate and BP

Subject questionnaire

Study eye photopic BCDVA (followed by fellow eye and then binocular BCDVA)

Slit lamp biomicroscopy

Corneal specular microscopy, if available

Intraocular pressure

Ophthalmoscopy

Adverse events

Study medication collected and new study medication dispensed

Subject diary review

5.4.10 Visit 10 (Stage 2 Follow-Up)/Week 36 ± 3 days

During the Week 36 Visit, the following safety measurements will be made:

Concomitant medications

Urine pregnancy test (for females of childbearing potential only)

Heart rate and BP

Subject questionnaire

Study eye photopic BCDVA (followed by fellow eye and then binocular BCDVA)

Slit lamp biomicroscopy

Corneal specular microscopy, if available

Intraocular pressure

Ophthalmoscopy

Adverse events

Study medication collected and new study medication dispensed

Subject diary review

5.4.11 Visit 11 (Stage 2 Follow-Up)/Week 42 ± 3 days

During the Week 42 Visit, the following safety measurements will be made:

Concomitant medications

Urine pregnancy test (for females of childbearing potential only)

Heart rate and BP

Subject questionnaire

Study eye photopic BCDVA (followed by fellow eye and then binocular BCDVA)

Slit lamp biomicroscopy

Corneal specular microscopy, if available

Intraocular pressure

Ophthalmoscopy

Adverse events

Study medication collected and new study medication dispensed

Subject diary review

5.4.12 Visit 12 (End of Study)/Week 48 ± 3 days

During the Week 48 Visit, the following safety measurements will be made:

Concomitant medications

Urine pregnancy test (for females of childbearing potential only)

Heart rate and BP

Subject questionnaire

Study eye photopic BCDVA (followed by fellow eye and then binocular BCDVA)

Slit lamp biomicroscopy

Corneal specular microscopy, if available

Intraocular pressure

Ophthalmoscopy

Adverse events

Study medication collected

Subject diary collected

5.4.13 Unscheduled Visits

An unscheduled visit may be any visit to the Investigator other than the specific visits requested in the protocol as possibly required for the subject's ophthalmic condition. The Investigator will perform all procedures necessary to evaluate the subject at these visits and record any AEs in the electronic Case Report Form (eCRF).

As noted in Section 5.11.4, every possible effort should be made by Investigators to assure that subjects who discontinue early from the study have a follow-up (FU) telephone call that includes assessments of AEs and concomitant medications.

5.4.14 Visit Variation

The Day 8 and Day 15 Visits may occur with a window of ± 1 day, and all subsequent visits may occur with a window of ± 3 days.

5.5 Measures Taken to Minimize/Avoid Bias

This is a randomized, placebo-controlled, double-masked, Phase 3 study. The placebo ophthalmic solution and the POS ophthalmic solution will be provided in identical packaging to ensure masking to Investigators, site staff, subjects, and Opus.

5.6 Study Medications

Study Medication Identification

The POS study medication is phentolamine mesylate; [redacted]

Established name	Phentolamine mesylate – parent phentolamine
CAS registry number	65-28-1 – parent 50-60-2
Chemical class	An alpha-adrenergic antagonist, it is a member of the following classes: imidazoles, of phenols, is a tertiary amino compound and a substituted aniline
[redacted]	[redacted]
[redacted]	[redacted]

Molecular weight (g/mol)	377.140 – parent 281.352
Drug name/formulation	POS/aqueous isotonic solution
Concentration active	Mesylate salt 1% – parent 0.75%
[redacted]	[redacted]
[redacted]	[redacted]
Storage requirements	Shipped and stored at site refrigerated (2°C to 8°C, 36°F to 46°F) until provided to the subject; then subject stores at room temperature (15°C to 25°C, 59°F to 77°F) Stored at the site, drug will be placed in a secured location (locked) with no access for unauthorized personnel

Formulation

POS (0.75% Phentolamine Ophthalmic Solution) is a clear, colorless to slightly brown, sterile, non-preserved, isotonic, buffered aqueous solution containing 1% phentolamine mesylate (equivalent to 0.75% phentolamine free base), mannitol, sodium acetate, and water for injection. Placebo for POS is a clear, colorless, sterile, non-preserved, isotonic, buffered aqueous solution containing mannitol, sodium acetate, and water for injection. The pH of both POS and its placebo is adjusted with NaOH (United States Pharmacopeia [USP]) and/or HCl (USP) to a pH range between 4.5 and 5.3.

5.6.1 Packaging and Labeling

The investigational products, POS and placebo, are packaged in a 0.5-mL low-density polyethylene Blow-Fill-Seal vial containing 0.3 mL solution for single-dose use. Five individual vials are included in a molded strip. Each strip containing 5 vials is wrapped with a multicolor "rainbow" aluminum foil overwrap that has been purged with nitrogen. The foil is impermeable to water and oxygen and will be labeled with an investigational label showing the study protocol number and other relevant information, including a statement "Caution – New Drug – Limited by Federal (US) Law to Investigational Use."

5.6.2 Storage of Study Medication and Dispensing

Prior to dispensing, all investigational material must be stored in a secure location with strictly limited access documented by signature of authorized persons who may dispense investigational materials.

Study medication (POS and its placebo) should be shipped and stored at the study site refrigerated (2°C to 8°C, 36°F to 46°F) until provided to the subject, and then the subject can store it at room temperature (15°C to 25°C, 59°F to 77°F). Study medication must not be frozen and must be protected from light.

5.6.3 Study Medication Administration

Study medication will be dispensed by the Investigator or designee at the site at Visit 1 (Screening/Baseline) and Visit 5 (End of Stage 1)/Week 6 during Stage 1 and at each visit except for Visit 12 (End of Study)/Week 48 during optional Stage 2.

The first dose of study medication will be administered in the clinic by site staff at Visit 1. If the subject wears contact lenses, they must be removed prior to dosing and not replaced until the next morning at the earliest.

During Stage 1, the subject will instill 1 drop of study medication QD OU in the evening near bedtime for approximately 6 weeks (± 3 days) beginning the evening of Day 2. On the evening before each of the remaining Stage 1 visits (Days 3, 8, and 15 and Week 6), subjects should dose study medication as close as possible to 12 hours prior to the scheduled time for those visits.

During optional Stage 2, the subject will continue to self-administer study medication QD OU in the evening near bedtime for an additional 42 weeks (± 3 days).

5.6.4 Study Medication Accountability

5.6.4.1 Receipt and Disposition of Study Medication

The Investigator or designee (eg, Study Coordinator or Pharmacist) will maintain a full accountability record for the study medication and will be responsible for recording the receipt, dispensing, and return of all supplies of the study medication using the inventories supplied by Opus and for monitoring dosing compliance using subject diaries. The Study Monitor will review dispensing and study medication accountability records during site visits and at the completion of the study and note any discrepancies.

Each subject will receive sufficient study medication for approximately the next 6 weeks; thus, subjects participating in optional Stage 2 will receive additional study medication at the Week 6, 12, 18, 24, 30, 36, and 42 Visits. A study medication box will be dispensed at Visit 1 (Screening/Baseline; Day 1). As described in Section 7.1, the subject will empty any remaining contents from the single-use vial following each instillation. The subject should then place the vial in the study medication box for return to the study site. All opened vials and any unopened study medication will be returned at Visit 5 (End of Stage 1)/Week 6 during Stage 1 and at each visit during optional Stage 2.

5.6.4.2 Return of Study Medication by Study Site

When the study is completed or is terminated by Opus, all study materials, including used and unused study medication vials, must be returned to Opus (or its designee) or destroyed under the direction of the same. All study medication accounting procedures must be performed before the study is considered completed. A final study medication disposition will be completed by the Study Coordinator.

5.7 Expected Duration of Subject Participation

The length of subject participation is approximately 6 weeks for Stage 1, with 5 clinic visits summarized below:

- Visit 1 (Screening/Baseline)/Day 1
- Visit 2 (Stage 1 Treatment)/Day 3
- Visit 3 (Stage 1 Treatment)/Day 8 (± 1 day)
- Visit 4 (Stage 1 Treatment)/Day 15 (± 1 day)

- Visit 5 (End of Stage 1)/Week 6 (± 3 days)

At Visit 5, subjects agreeing to continue to Stage 2 will be reconsented. Optional Stage 2 will continue for approximately an additional 42 weeks, with 7 clinic visits summarized below:

- Visit 6 (Stage 2 FU)/Week 12 (± 3 days)
- Visit 7 (Stage 2 FU)/Week 18 (± 3 days)
- Visit 8 (Stage 2 FU)/Week 24 (± 3 days)
- Visit 9 (Stage 2 FU)/Week 30 (± 3 days)
- Visit 10 (Stage 2 FU)/Week 36 (± 3 days)
- Visit 11 (Stage 2 FU)/Week 42 (± 3 days)
- Visit 12 (End of Study)/Week 48 (± 3 days)

5.8 Randomization and Procedure for Breaking the Code

A randomization code for allocating subjects to each treatment arm will be prepared by a masked biostatistician not connected with the study. Subjects will be stratified by iris color (light/dark irides).

At the initiation of study-related procedures, every subject screened is assigned a subject number within numerical order within each site. Once a subject is qualified for the study, the subject is assigned a ***randomization number*** in the order provided by the biostatistician.

Study medications will be masked to Investigators, site staff, study subjects, the contract research organization (CRO), and Opus. Only in case of medical emergency or occurrence of SAEs will the randomization code be unmasked by the Medical Monitor and made available to the Investigator, Opus, and/or other personnel involved in the monitoring or conduct of this study.

5.9 Collection of Data

Study-specific data that have been outlined in the protocol will be entered into the clinical database by individual(s) designated by the Investigator. Data will be verified electronically using a series of online programmed edit checks that have been created by the Clinical Data Manager and programmed by the Clinical Data Programmer or designee. Data discrepancies will be brought to the attention of the clinical team and investigated by the Study Monitor and site staff. Study Monitors will review and verify all data collected in the eCRF against any applicable source documentation during remote review or scheduled monitoring visits. The Study Monitor will work closely with the site staff to address any discrepancies found so that proper resolutions can be made and documented in the clinical database. An audit trail within the system will track all changes made to the data.

5.10 Completed Subject

A completed subject is defined as one who completes all planned procedures through the end of Visit 5 (End of Stage 1)/Week 6 (for those who did not consent to participate in optional Stage 2) or through the end of Visit 12 (End of Study)/Week 48 (for those who did consent to participate in optional Stage 2).

5.11 Non-completing Subject

A non-completing subject is defined as one who exits the study by their own volition or at the discretion of the Investigator and/or the Medical Monitor prior to completing all of the study procedures required in this protocol. Any subject may decide to voluntarily withdraw from the study at any time without prejudice.

5.11.1 Study Medication Discontinuation

Study medication may be discontinued for the following reasons:

- **Adverse event:** Adverse events include clinically significant laboratory abnormalities and intercurrent diseases reported by the subject or observed by the Investigator, with documentation on the eCRF
- **Death:** If a subject dies, the AE(s) that caused the death should be documented on the eCRF and be noted as serious and fatal
- **Disallowed concurrent medication:** Any medication not allowed by the protocol would be a protocol violation
- **Lack of efficacy:** A subject may elect to discontinue participation in the study for a perceived lack of efficacy
- **Investigator decision:** A subject may be discontinued for reasons other than those bulleted previously if the Investigator thinks it is not in the best interest of the subject to continue
- **Other:** If there is any other reason for subject discontinuation, this should be documented on the eCRF

5.11.2 Reasons for Withdrawal From Study

- Subject withdraws consent
- Subject withdraws due to an AE
- Subject is lost to follow-up
- Subject withdraws for other reason

5.11.3 Entire Study Terminated

The entire study may be terminated by Investigators or Opus. Prompt, written notice of reasonable cause to the other party (Opus or Investigators, respectively) is required.

Prompt notice to the Institutional Review Board (IRB) and to regulatory authorities is also required.

5.11.4 Actions After Discontinuation

All subjects who discontinue study medication due to a report of an AE **must** be followed up and provided appropriate medical care until their signs and symptoms have remitted or stabilized or until medical assessments have returned to acceptable or pre-study limits.

For any subject who chooses to withdraw consent or who is noncompliant, every possible effort should be made by the Investigator to assure the Visit 4/Day 15 mLCVA and other measurements are assessed prior to discontinuation, in addition to a FU telephone call that includes assessments for AEs and concomitant medications.

5.12 Completed Study

The study is completed when all randomized subjects have completed or discontinued the study, all eCRFs have been completed, and all data have been entered into the database. Final database lock will occur after the last randomized subject completes the last visit, all data have been entered, and all queries are resolved.

6 SUBJECT INCLUSION AND EXCLUSION CRITERIA

6.1 Subject Inclusion Criteria

Subjects must meet all of the following criteria:

1. Males or females ≥ 18 years of age
2. Previous history of refractive surgery (eg, photorefractive keratectomy [PRK], laser-assisted in situ keratomileusis [LASIK], small-incision lenticule extraction [SMILE], and radial keratotomy [RK]) and have subject-reported night vision disturbances (eg, glare, halos, and/or starbursts). Symptoms must have been first noted within 2 months following refractive surgery
3. Able to independently comply with all protocol-mandated procedures and to attend all scheduled office visits
4. Able and willing to give written consent to participate in this study
5. Able to self-administer study medication

Inclusion criteria #6, #7, and #8 must all be met in the same eye:

6. Pupil diameter ≥ 5 mm under mesopic conditions in at least 1 eye. This test may be repeated once, following an additional 5 min of dark adaptation to the mesopic light conditions if the initial results do not meet this criterion
7. Mesopic low-contrast VA ≤ 30 ETDRS letters (20/63 Snellen or worse) in at least 1 eye using the Precision Vision Illuminator Cabinet with 5% translucent contrast chart and a mesopic filter at 4 m
8. [redacted]

6.2 Subject Exclusion Criteria

Excluded from the study will be individuals with any of the following characteristics:

Ophthalmic (in either eye):

1. Prior unresolved dry eye diagnosis, taking prescription drops for dry eye, or taking artificial tear drops routinely for dry eye
2. Prior history of fluctuating vision
3. Clinically significant ocular disease as deemed by the Investigator (eg, untreated visually significant cataract, glaucoma, corneal edema, uveitis, severe keratoconjunctivitis sicca, retina degeneration, loss of visual field due to glaucoma or stroke, branch retinal vein occlusion, retina flare) that might interfere with the study
4. History or presence of corneal endothelial dystrophy (eg, Fuchs' dystrophy or presence of guttae)
5. Known hypersensitivity to any topical alpha-adrenoceptor antagonists
6. Known allergy or contraindication to any component of the vehicle formulation

7. History of cauterization of the punctum or punctal plug (silicone or collagen) insertion or removal
8. Pseudophakic subjects with extended depth-of-focus or multifocal intraocular lenses (IOLs)
9. Ocular trauma, ocular surgery (eg, IOLs), or laser procedure (eg, LASIK, PRK, SMILE, and RK) within 6 months prior to Screening
10. Use of any topical prescription or over-the-counter (OTC) ophthalmic medications of any kind (including artificial tear drops) within 7 days prior to Screening until study completion, with the exception of lid scrubs with OTC products (eg, OCuSOFT® lid scrub, SteriLid®, baby shampoo, etc.)
11. Recent or current evidence of ocular infection or inflammation (such as current evidence of clinically significant blepharitis, conjunctivitis, or a history of herpes simplex or herpes zoster keratitis at Screening). Subjects must be symptom free for at least 7 days prior to Screening
12. History of diabetic retinopathy, diabetic macular edema, or dry or wet macular degeneration
13. History of any traumatic (surgical or nonsurgical) or nontraumatic condition affecting the pupil or iris (eg, irregularly shaped pupil, neurogenic pupil disorder, iris atrophy, iridotomy, iridectomy, etc.)
14. Unwilling or unable to discontinue use of contact lenses at least 1 hour prior to Screening for soft contact lenses or at least 8 hours prior to Screening for hard gas-permeable contact lenses, and at least 8 hours (for both types of lenses) prior to all other office visits
15. Previously undiagnosed dry eye, at the determination of the Investigator. Dry eye diagnosis should be based on one of the following dry eye test results: tear break-up time < 5 seconds, or corneal fluorescein staining ≥ Grade 2 in the inferior zone or ≥ Grade 1 in the central zone using the National Eye Institute scale

Systemic:

16. Known hypersensitivity or contraindication to alpha- and/or beta-adrenoceptor antagonists (eg, chronic obstructive pulmonary disease or bronchial asthma; abnormally low BP or HR; second- or third-degree heart blockage or congestive heart failure; or severe diabetes as defined below)
    a. Predisposition to severe hypoglycemia (2 or more serious hypoglycemic episodes requiring assistance within 12 months prior to Screening)
    b. Any hospitalization or emergency room visit due to poor diabetic control within 6 months prior to Screening
    c. Currently untreated diabetes mellitus or previously untreated subjects who initiated oral anti-diabetic medication or insulin within 3 months prior to Screening
    d. Any sign of diabetic retinopathy in either eye

17. Clinically significant systemic disease (eg, severe diabetes as previously defined, myasthenia gravis, cancer, hepatic, renal, endocrine, or cardiovascular disorders) that might interfere with the study
18. Initiation of treatment with or any changes to the current dosage, drug, or regimen of any systemic adrenergic or cholinergic drugs within 7 days prior to Screening or during the study
19. Participation in any investigational study within 30 days prior to Screening or during the study
20. Females of childbearing potential who are pregnant, nursing, planning a pregnancy during the study, or not using a medically acceptable form of birth control. Acceptable methods include the use of at least one of the following: intrauterine device, hormonal (oral, injection, patch, implant, ring), barrier with spermicide (condom, diaphragm), or abstinence. A female is considered to be of childbearing potential unless she is 1 year postmenopausal or 3 months post−surgical sterilization. All females of childbearing potential including those post−tubal ligation must have a negative urine pregnancy test result at each visit
21. Resting HR outside 50 to 110 beats per min (bpm) at Screening. Heart rate may be repeated **only once** if outside the specified range, following at least a 5-min rest period in the sitting position
22. Hypertension with resting diastolic BP > 105 mmHg or systolic BP > 160 mmHg at Screening. Blood pressure may be repeated **only once** if outside the specified range, following at least a 5-min rest period in the sitting position

7 TREATMENT OF SUBJECTS

Approximately 200 subjects ≥ 18 years of age will be randomized in a 1:1 ratio to 1 of 2 treatment arms (POS or placebo). Randomization will be stratified by iris color (light/dark irides).

7.1 Treatment Adherence

All subjects must be instructed on the importance of following the QD regimen and maintaining their dosing diaries. At Visit 1 (Screening/Baseline)/Day 1, subjects will receive 1 drop of study medication OU instilled by site staff. On Day 2, subjects will initiate self-administration of 1 drop of study medication OU in the evening near bedtime. Subjects will repeat this instillation, using 1 vial per day, for a total of 6 weeks over the course of the study. Subjects are to empty any remaining contents from the single-use vial following each instillation and bring all empty, opened vials as well as any unopened study medication in the study medication box to the study site at Visit 5 (End of Stage 1)/Week 6 during Stage 1 and at each visit during optional Stage 2. Treatment adherence will be measured by counting the vials at the start of the study and those remaining at Visit 5 (End of Stage 1)/Week 6 for Stage 1 and at each visit for optional Stage 2. Subjects will also bring their diary to each post-baseline visit and dosing will be reviewed by site staff.

7.2 Concomitant Medications

As noted in the exclusion criteria (Section 6.2), use of any topical prescription or OTC ophthalmic medications of any kind within 7 days prior to Screening is prohibited, with the exception of lid scrubs with OTC products (eg, OCuSOFT® lid scrub, SteriLid®, baby shampoo, etc.). Many drugs, both prescription and OTC, contain active ingredients that can affect PD. This includes many eye drops such as Visine® that are used to reduce redness, most cough or cold preparations, antihistamines and bronchodilators, most nasal drops, most BP medications, many drugs used for migraines, and many other products.

Additionally, initiation of treatment with or any changes to the current dosage, drug, or regimen of any systemic adrenergic or cholinergic drugs (Appendix 3) within 7 days prior to Screening or during the study is prohibited. However, a subject can be treated with a systemic adrenoceptor antagonist, for example, as long as the particular agent and its dose and regimen had been consistent for the 7 days prior to Screening, and there is no reason to believe that alteration will be necessary at some point later during the study.

If there is any question about whether a medication is acceptable, the Medical Monitor should be consulted before proceeding.

Use of all medications will be documented on the appropriate eCRF. Judgment of continued study participation by the subject and inclusion of this subject's subsequent visits in the safety and efficacy analysis will be made by Opus.

All medications taken by the subject within 30 days prior to Screening and during the study will be documented on the eCRF. The name of the drug, dose, route of administration, duration of treatment, and indication will be recorded for each medication. For combination products (eg, Contac®, Cosopt®), the brand name is required. For non-combination products, the generic name is desired. The use of routine ophthalmic diagnostic pharmaceutical agents (eg, fluorescein and local anesthetic) will be allowed and

should be documented. Any change in dosing parameters should also be recorded in the eCRF.

8 ASSESSMENT OF EFFICACY

8.1 Specification of the Efficacy Parameters

The primary efficacy endpoint is the percent of subjects with ≥ 15 ETDRS letters improvement in the study eye in mLCVA compared to Baseline (Day 1 pre-dose) at Day 15 for the mITT Population. Other efficacy endpoints can be found in Sections 5.2 and 5.3.

8.2 Assessing, Recording, and Analyzing Efficacy Parameters

Visual acuity, PD, and subject questionnaire (Appendix 2) will be measured at each visit in Stage 1, as described in Section 5.4.

The subject should be in the same room for all assessments, and every effort will be made to have the same staff member perform the measurements at all visits.

The study eye, fellow eye, and binocular measurements will be evaluated at all appropriate assessments. All of the efficacy endpoints will be analyzed overall and by light/dark irides at all time points. The mLCVA endpoints will be analyzed by baseline PD (≥ 6, ≥ 7, and ≥ 8 mm).

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

The assessment of safety and tolerability is a primary objective of this study and will be evaluated by:

- Best-corrected distance VA
- Biomicroscopy of the anterior segment, including evaluation of cornea, conjunctiva, and anterior chamber
- Corneal specular microscopy, if available
- Intraocular pressure
- Ophthalmoscopy direct or indirect fundus exam without dilation, including optic nerve, macula, vessels, and periphery
- Heart rate and BP
- Adverse events

9.2 Adverse Events and Serious Adverse Events

All AEs and SAEs that occur following consent and until the final study visit should be collected and recorded on the AE or SAE eCRF page. Only treatment-emergent adverse events (TEAEs)/treatment-emergent adverse reactions will be summarized (Section 10.3.7).

All AEs/adverse reactions occurring during the study (ie, once the subject has signed the informed consent) **must** be documented, regardless of the assumption of causal relationship, on the respective eCRF. All TEAEs/adverse reactions must be documented from the time the subject receives the study medication until the subject's participation in the study has been completed. If a subject has ongoing AEs/adverse reactions at the time of study completion or discontinuation from the study, the ongoing AEs/adverse reactions **must** be followed up and provided appropriate medical care until the signs and symptoms have remitted or stabilized or until medical assessments have returned to acceptable or pre-study limits.

Documentation of AEs/adverse reactions includes start date and end date, severity, relationship to study medications, action(s) taken, seriousness, and outcome.

9.2.1 Adverse Event Definitions

The following definitions of terms apply to this section:

Adverse event. An AE is any untoward medical occurrence associated with the use of a study medication in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory findings), symptom, or disease temporally associated with the use of the study medication, whether or not related to the study medication. Study medication includes the investigational

product under evaluation and the comparator product or vehicle placebo given or administered during any phase of the study.

Medical conditions/diseases present before starting the investigational treatment are only considered AEs if they worsen after starting the investigational treatment. Abnormal test results constitute AEs only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

The occurrence of AEs should be sought by open-ended questioning of the subject at each visit during the study. At each clinic assessment/visit, study personnel should ask the following question: "Have you had any problems since your last assessment/visit?". Adverse events also may be detected when they are volunteered by the subject during or between visits or through study assessments.

Life-threatening AE or life-threatening suspected adverse reaction. An AE or suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator or Opus, its occurrence places the subject at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious AE or serious suspected adverse reaction. An AE or suspected adverse reaction is considered ''serious'' if, in the view of either the Investigator or Opus, it results in any of the following outcomes:

- Death
- Life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital anomaly/birth defect
- Other medically important serious event

Important medical events that may not result in death, be 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.

Treatment on an outpatient emergency basis that does not result in hospital admission, or a hospitalization that is elective or is a preplanned treatment for a pre-existing condition that is unrelated to the medication under study and has not worsened since the start of the study is not considered an SAE.

Suspected adverse reaction means any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of Investigational New Drug safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the AE. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a drug.

Unexpected AE or unexpected suspected adverse reaction. An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the IB or is not listed at the specificity or severity that has been observed; or, if an IB is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. "Unexpected," as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

The study medication relationship for each AE/adverse reaction should be determined by the Investigator using these explanations:

- Not related
- Unlikely related
- Possibly related
- Probably related
- Definitely related
- Unknown

Unless the relationship is considered to be "Not related" or "Unlikely related" and there is any valid reason, even if undetermined, for suspecting a possible cause-and-effect relationship between the study medication and the occurrence of the AE, then the AE should be considered "related".

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

- 1 = Mild: present, but not distressing, and no disruption of normal daily activity
- 2 = Moderate: discomfort sufficient to reduce or affect normal daily activity
- 3 = Severe: incapacitating, with inability to work or perform normal daily activity

A change in severity for a reported AE will require an end date for the previous severity and a new start and end date for the new severity. For example, a change in severity may go from mild to severe or from severe to moderate. In either case, the start or end times/dates should be recorded.

The term "severe" is used to describe the intensity of an event/reaction; the event/reaction itself may be of relatively minor medical significance (such as a severe headache). This is not the same as a "serious" AE, which is based on a subject/event outcome or action criteria usually associated with events that pose a threat to the subject's life or vital functions. "Seriousness" (NOT severity) serves as a guide for defining regulatory reporting obligations.

Should a pregnancy occur, it must be reported and recorded on the pregnancy form and emailed to LYNX2_Safety@Oculoscr.com, as well as recorded in the eCRF. Pregnancy in itself is not regarded as an AE unless there is a suspicion that an investigational product may have interfered with the effectiveness of a contraceptive medication.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) must be followed up and documented even if the subject was discontinued from the study.

Action taken in response to an AE is coded as:

- Dose increased: An indication that a medication schedule was modified by addition; either by changing the frequency, strength, or amount
- Dose not changed: An indication that a medication schedule was maintained
- Dose reduced: An indication that a medication schedule was modified by subtraction, either by changing the frequency, strength, or amount
- Dose interrupted: An indication that a medication schedule was modified by temporarily terminating a prescribed regimen of medication
- Drug withdrawn: An indication that a medication schedule was modified through termination of a prescribed regimen of medication
- Not applicable: Determination of a value is not relevant in the current context
- Unknown: Not known, not observed, not recorded, or refused

Additional other action taken:

- Concomitant medication
- Hospitalization

Outcome of an AE is coded as:

- Fatal: The termination of life as a result of an AE
- Not recovered/not resolved: One of the possible results of an AE outcome that indicates that the event has not improved or the subject has not recuperated
- Recovered/resolved: One of the possible results of an AE outcome that indicates that the event has improved or the subject has recuperated
- Recovered/resolved with sequelae: One of the possible results of an AE outcome in which the subject recuperated but retained pathological conditions resulting from the prior disease or injury
- Recovering/resolving: One of the possible results of an AE outcome that indicates that the event is improving or the subject is recuperating
- Unknown: Not known, not observed, not recorded, or refused

In previous clinical studies of POS, the most frequently reported AE was conjunctival hyperemia. Investigators are cautioned to use the appropriate verbatim term on the AE form to describe this observation:

- Redness related to instillation that is transient (ie, is no longer present 2 hours after instillation) = "conjunctival erythema upon instillation"
- Redness that is NOT transient (ie, is present ≥ 2 hours after instillation) = "conjunctival hyperemia"

Expedited reporting of serious and unexpected AEs: All SAEs (related and unrelated) will be recorded following subject signature of the informed consent and until Visit 5 (End of Stage 1)/Week 6 for those subjects not participating in optional Stage 2 and until Visit 12 (End of Study)/Week 48 for those subjects participating in optional Stage 2. Any SAEs "suspected" to be related to the study medication and discovered by the Investigator at any time **after** the study should be reported.

Any SAE that occurs must be reported to the CRO within 24 hours of its occurrence or within 24 hours of learning of its occurrence. Recurrent episodes, complications, or progression of the initial SAE must be reported to the CRO as follow-up to the original episode within 24 hours of the Investigator receiving the information. Information about all SAEs will be collected and recorded on the SAE form. All pertinent medical records and information collected during the treatment and follow-up of the subject should be maintained at the site with a copy emailed to [redacted]. The Investigator must assess the SAE relationship and complete the SAE form. The CRO may request additional information. Follow-up information (eg, discharge summary) will be retained in the subject's chart and a copy will be emailed to [redacted]. In addition, all SAEs should be recorded on the AE eCRF page with the serious question marked "Yes".

It is the Investigator's responsibility to notify the approving IRB of any SAEs on a timely basis as instructed by Opus following Opus's determination of causality. All subjects who experience an SAE should be followed clinically and undergo the appropriate diagnostic evaluations until stabilization or resolution of the event. Opus will report all SAEs to the FDA on the appropriate schedule depending if the event is drug related or not drug related and expected or unexpected (based on the available information in the IB).

Any death occurring during the study and FU period must be reported as an SAE. For any death occurring through the end of the study, regardless of the degree of relationship to study medication, the SAE resulting in the death must be reported to the CRO. A death occurring after completion of the study that is not reasonably associated with study medication administration does not require completion of the SAE form.

9.2.2 Follow-Up of Subjects After Adverse Events

If an AE/adverse reaction occurs, the Investigator will institute support and/or treatment as deemed appropriate. All AEs ongoing at the time of the last visit or discontinuation from the study will be followed up until the AE/adverse reaction is resolved or stabilized, the subject is lost to follow-up, or there is other resolution to the event.

10 STATISTICS

10.1 Sample Size

A total of approximately 200 subjects will be enrolled and randomized into the study in a 1:1 ratio to 1 of the 2 treatment arms (POS or placebo), with a stratification by light/dark irides.

The primary efficacy endpoint will be met if subjects show a positive effect for POS (α = 0.05 significance, 2-sided) at Day 15 in the study eye. A total of 200 subjects will provide 89% power to detect a difference of 16% between the POS and placebo groups in percent of subjects with ≥ 15 ETDRS letters improvement in monocular mLCVA at Day 15 compared to Baseline. [redacted]

To test for tachyphylaxis, a sample size of 100 subjects receiving POS provides greater than 90% power (α = 0.05 significance) to detect a 5-letter or more inferiority of the change in monocular mLCVA after 6 weeks of daily dosing compared to the best change in mLCVA during the first month of the study, assuming a standard deviation of 9 letters.

10.2 Analysis Populations

Modified Intention-to-Treat Population: The mITT Population will include all randomized subjects who received at least 1 dose of study medication. The mITT Population will be used for the primary endpoint analysis and to analyze efficacy endpoints, with subjects included in their randomized treatment regardless of the treatment they actually received.

Per Protocol (PP) Population: The PP Population will include all subjects in the mITT Population who received at least 80% of expected doses of study medication through Day 15, have a Baseline and a Day 15 mLCVA measurement, and have no major protocol deviations considered to have significant impact on treatment outcome. The PP Population will be used to analyze efficacy endpoints, with subjects included in their randomized treatment group regardless of the treatment they received.

All Randomized Population (ARP): The ARP will include all randomized subjects. This population is also known as the Intention-to-Treat (ITT) Population.

Safety Population: The Safety Population will include all randomized subjects who have received at least 1 dose of study medication. The Safety Population will be used to summarize safety variables, using the actual treatment a subject received.

10.3 Statistical Methods

10.3.1 General Considerations

All continuous variables will be summarized by treatment using descriptive statistics (n, mean, median, standard deviation, minimum, and maximum). All categorical variables will be summarized by treatment using counts and proportions.

All study data will be listed by treatment group and subject.

All statistical tests will be performed using a significance level of 5% (two-tailed). The p-values for the analysis of secondary efficacy endpoints will be considered descriptive.

10.3.2 Demographic and Baseline Characteristics

Subject demographic data and baseline characteristics will be tabulated and summarized descriptively by treatment group and overall. The demographic data and baseline characteristics will be summarized for the mITT Population, PP Population, Safety Population, and ARP. The demographics consist of age (year), sex, race, ethnicity, study eye (right or left), and irides type (light or dark). These data will also be provided in by-subject listings.

10.3.3 Subject Disposition

A summary of the analysis sets includes the number and percentage of subjects by treatment group and overall for the following categories: subjects in the ARP, subjects in the Safety Population, subjects in the mITT Population, and subjects in the PP Population. All percentages will be based on the number of subjects in the ARP. End-of-study information will also be summarized in this table, including the number of subjects completing Week 6, the number of subjects completing the study, the number of subjects who prematurely discontinued the study with reasons for withdrawal, and the number of subjects who prematurely discontinued the study medication with reasons for study medication discontinuation. These data will also be provided in by-subject listings.

10.3.4 Medical History and Prior/Concomitant Medications

Medical history will be coded using the latest version of Medical Dictionary for Regulatory Activities (MedDRA) and will be summarized for all subjects by treatment group and overall. Medical history will be provided in by-subject listings.

Prior medications are defined as any medication that has a start and stop date prior to the day of first exposure to any study medication. The total number of prior medications and the number and percentages of subjects with at least 1 prior medication will be summarized by treatment.

The total number of concomitant medications and the number and percentages of subjects with at least 1 concomitant medication will be summarized by treatment. All summaries will be performed using the Safety Population.

10.3.5 Estimands for Efficacy

The primary efficacy estimand, corresponding to the primary efficacy endpoint, is defined by the following components:

1. The *population* is restricted to the mITT Population
2. The *variable* for the primary endpoint is percent of subjects with ≥ 15 ETDRS letters improvement in the study eye in mLCVA compared to Baseline (Day 1 pre-dose) at Day 15
3. The *intercurrent events* of treatment discontinuation or use of prohibited concomitant medications will be handled according to the treatment policy strategy, irrespective of premature discontinuation of randomized treatment

4. The *population-level summary* is the difference in percent of subjects with ≥ 15 ETDRS letters improvement in the study eye in mLCVA compared to Baseline (Day 1 pre-dose) at Day 15, analyzed using a logistic regression model with treatment and light/dark irides as factors and the baseline mLCVA as a covariate
5. *Treatment:* There will be 1 active treatment arm (POS) and 1 control arm (placebo)

Secondary efficacy estimands will have the same population, intercurrent events, and treatment as the primary estimand. For secondary categorical endpoints, the population-level summary will also match the primary estimand; only changes to the variable (eg, visit, type of assessment) will be required to match the particular secondary endpoint.

Secondary efficacy estimands for continuous endpoints will also vary depending on the assessment, visit, and time point. For change from baseline endpoints, the population-level summary is the change from Baseline in the assessment at the required visit, analyzed using analysis of covariance (ANCOVA) with treatment and light/dark irides as factors and the baseline assessment as a covariate.

The following is an example of a secondary efficacy estimand with a change from baseline endpoint:

1. The *population* is restricted to the mITT Population
2. The *variable is* change from Baseline (Day 1 pre-dose) in mLCVA in the study eye at Day 15
3. The *intercurrent event* of treatment discontinuation or use of prohibited concomitant medications will be handled according to the treatment policy strategy, irrespective of premature discontinuation of randomized treatment
4. The *population-level summary* is the change from Baseline in mLCVA in the study eye at Day 15, analyzed using ANCOVA with treatment and light/dark irides as factors and the baseline mLCVA as a covariate
5. *Treatment:* There will be 1 active treatment arm (POS) and 1 control arm (placebo)

10.3.6 Analysis of Efficacy

Unless otherwise noted, efficacy will be assessed using the mITT and PP Populations, with subjects included in the treatment arm into which they were randomized. For the analysis of the primary efficacy endpoint, imputation will be performed for missing observations if applicable; details will be provided in the Statistical Analysis Plan (SAP). If the analysis using the mITT Population shows a positive effect for POS at the 0.05 level of significance, the primary endpoint will be considered met. The primary efficacy endpoint is the percent of subjects with ≥ 15 ETDRS letters improvement in the study eye in mLCVA compared to Baseline (Day 1 pre-dose) at Day 15. The primary efficacy endpoint will be analyzed using a logistic regression model with treatment and light/dark irides as factors and the baseline mLCVA as a covariate. The percentage of subjects in each treatment arm meeting the criteria, the difference in proportions with 95% confidence interval (CI), and p-value will be provided.

In addition, the primary efficacy endpoint will be analyzed by light/dark irides using the same model indicated above but without irides as a factor. For these subgroup analyses, observed case data only will be used; that is, missing values will not be imputed. The primary efficacy endpoint will also be analyzed by subgroups based on age, gender, race, and ethnicity; full details will be presented in the SAP.

For all efficacy endpoints, Baseline values are defined as the screening assessment values on Visit 1/Day 1 (pre-dose, if applicable).

Other efficacy endpoints are indicated in Sections 5.2 and 5.3.

Each of the continuous secondary efficacy endpoints will be analyzed using ANCOVA with change from Baseline as the dependent variable, treatment and light/dark irides as factors, and the respective baseline value included as the covariate. The output from each ANCOVA will include the least-squares (LS) mean and standard error for both treatment arms, along with the placebo-corrected LS mean, its 95% CI, and associated p-value.

For each of the secondary endpoints related to percent of subjects achieving certain criteria, the analysis will be performed using a logistic regression model with treatment and light/dark irides as factors and the baseline mLCVA as a covariate. For each analysis, the percentage of subjects in each treatment arm meeting the criteria, the odds ratio with 95% CI, and p-value will be provided.

In addition, each secondary efficacy endpoint will be analyzed by light/dark irides using the same model indicated above but without irides as a factor.

A hierarchical analysis will be used to evaluate primary and secondary efficacy endpoints to ensure that the family of tests in the hierarchy will simultaneously be tested at the 0.05 level of significance.

Tachyphylaxis will be assessed for mLCVA by comparing change from Baseline at Week 6 in the POS group to the best change from baseline achieved during the first month of treatment for mLCVA.

Questions from the subject questionnaire will be summarized categorically for each treatment group and visit.

10.3.7 Analysis of Safety

Safety will be assessed using the Safety Population, with subjects included in the treatment arm they actually received, regardless of their randomized treatment. Observed case data will be used; no imputation will be performed for missing safety data.

Descriptive statistics of observed values will be presented for vital sign data at each visit including systolic BP (mmHg), diastolic BP (mmHg), and HR (bpm) by treatment group and overall. Changes from Baseline (Day 1) to each scheduled post-baseline time point will be presented.

Observed values and change from Baseline in IOP will be summarized descriptively using counts and percentages for each treatment group at each visit for the observed value and change from Baseline (Day 1). Separate summaries will be created for the study eye, the fellow eye, and the best of either eye.

Verbatim descriptions of AEs will be coded using MedDRA. Only TEAEs (those that occur after the first dose of study medication *or increasing in severity after initiation of study medication*) will be summarized. Treatment-emergent AEs and SAEs will be summarized by treatment, system organ class (SOC) and preferred term, severity, and relationship to study medication. Deaths, withdrawal from study medication due to AEs, and withdrawal from the study due to AEs will each be summarized by treatment. Note that in MedDRA, ocular events are coded to the SOC of "special senses". Thus, using SOC in the summaries will provide a separation of ocular and non-ocular AEs.

All safety data will be provided in by-subject listings.

10.4 Interim Analyses

The primary analysis of efficacy data will be conducted after all subjects complete Week 6 of the study. All efficacy and safety endpoints through Week 6, as well as the data for tachyphylaxis, will be analyzed. An additional interim analysis of safety endpoints will be conducted after all Stage 2 subjects complete Week 24 of the study.

For the interim analyses, Sponsor personnel who have access to individual treatment assignments will be kept to a minimum. In particular, Investigators and investigational site staff will remain masked to individual treatment assignments until database lock for final analysis.

10.5 Final Analysis

The final analysis will be conducted after all subjects have completed the study.

10.6 Procedure for Accounting for Missing, Unused, or Spurious Data

For the analysis of the primary efficacy endpoint, imputation will be performed for missing data; details of the imputation, if performed, will be included in the SAP. For the analysis of the other efficacy endpoints, only observed case data will be used. For the summarization of safety data, only observed case data will be used.

10.7 Procedure for Reporting Deviations From the Statistical Plan

Any deviations from the SAP will be described and a justification given in the final Clinical Study Report.

11 DIRECT ACCESS TO SOURCE DATA AND DOCUMENTS

The Investigator will permit study-related monitoring visits, audits, IRB review, and regulatory inspection(s) by providing direct access to source data and documents.

12 QUALITY CONTROL AND QUALITY ASSURANCE

The progress of the study will be monitored by onsite, written, and telephone communications between personnel at the Investigator's site and the Medical Monitor. Should the Coronavirus Disease 2019 (COVID-19) pandemic restrict Study Monitors from traveling to a site, remote review will be conducted to the extent possible, while still ensuring the study is monitored appropriately per applicable regulations and guidelines. The Investigator will allow Opus, the Study Monitor, and the Medical Monitor to inspect all eCRFs, subject records (source documents), signed ICFs, and records of study medication receipt, storage, preparation, disposition, and regulatory files related to this study.

13 ETHICAL CONSIDERATIONS AND GOOD CLINICAL PRACTICE COMPLIANCE

13.1 Good Clinical Practice Compliance

The proposed study is subject to all applicable governmental rules and regulations concerning the conduct of clinical trials on human subjects. This includes, but is not necessarily limited to, the approval of IRBs, the Helsinki Declaration, US FDA law, International Council for Harmonisation (ICH) Good Clinical Practice (GCP) guidelines, obtaining prospective informed consent, monitoring of the conduct of the study and the completeness of the eCRFs by Opus or its designee(s), and appropriate record retention by the Investigator.

13.2 Institutional Review Board

This protocol, materials used to recruit subjects, and materials used to document consent must be approved by the IRB prior to initiation of the study. Written IRB approval must adequately identify the protocol and informed consent. In addition to approving the protocol, the IRB must also approve the subject information and consent form, as well as any advertising tools that will be used for the study. Copies of all approved materials, all correspondence with the IRB, and written approval from the IRB must be made available to Opus, *prior* to the start of subject enrollment into the study.

13.3 Protocol Deviations/Violations

The Investigator should not deviate from the requirements of this protocol without prior written approval of the Medical Monitor or Sponsor, except in the event of a medical emergency.

A reportable protocol deviation is defined as nonadherence to the protocol that involves inclusion/exclusion criteria; affects subject safety, rights, or welfare; or has the potential to affect the integrity of the data. Examples of major protocol deviations include study enrollment by ineligible subject, loss of key data such as equipment malfunction (eg, pupillometer), and/or use of a prohibited medication during the study.

All protocol deviations will be reported by entering the event in the appropriate eCRF page. Protocol deviations should be reported to the IRB in accordance with IRB-specific guidelines. If there is any question as to whether the deviation is reportable, Opus or its designee and the IRB should be contacted.

All changes to the protocol will be made by the Sponsor or designee as an approved amendment to the protocol, submitted to the FDA, and approved by the IRB prior to implementation.

Changes implemented without prior approval will be considered protocol violations.

13.4 Informed Consent Requirements

Written informed consent will be obtained from each subject. A copy of the signed and dated consent document will be given to each subject. The original signed and dated ICF must be maintained in the study files at the Investigator’s site.

The Investigator is responsible for ensuring that no subject is subject to any study-related examination or activity before that subject has given informed consent. The subject must give written consent after the receipt of detailed information. The verbal explanation will cover all the elements specified in the written information provided for the subject.

It should be emphasized that the subject is at liberty to withdraw consent to participate at any time, without penalty or loss of benefits to which the subject is otherwise entitled. Subjects who refuse to give, or withdraw, written informed consent may not be included or continued in this study, but this will not impact their subsequent care.

The Investigator will inform the subject of the aims, methods, anticipated benefits, and potential hazards of the study, including any discomfort it may entail. The subject must be given every opportunity to clarify any points he/she does not understand and, if necessary, ask for more information. At the end of the interview, the subject may be given time to reflect, if this is required, or if the subject requests more time. Subjects who wish to participate in the study will be required to sign and date the ICF.

A copy of the signed and dated ICF will be given to each subject. The original signed and dated ICF must be maintained in the study files at the Investigator's site. Signed informed consent must be obtained prior to the conductance of any study procedures.

14 DATA HANDLING AND RECORD KEEPING

All procedures for the handling and analysis of data will be conducted using good computing practices meeting ICH and US FDA guidelines for the handling and analysis of data for clinical trials.

14.1 Data Entry

Study-specific data that have been outlined in the protocol will be entered into the clinical database by individual(s) designated by the Investigator.

14.2 Data Quality Control and Reporting

Data are verified electronically using a series of programmed edit checks that have been created by the Clinical Data Manager and programmed by the Clinical Data Programmer or designee. Data discrepancies will be brought to the attention of the clinical team and investigated by the Clinical Research Associate (CRA) and site staff. Clinical Research Associates will review and verify all data collected in the eCRF against source documentation during scheduled monitoring visits. The CRA will work closely with the site staff to address any discrepancies that have been found so that proper resolutions can be made and documented in the clinical database. An audit trail within the system will track all changes made to the data.

14.3 Archiving of Data

Archived versions of the database will be saved by Opus consistent with ICH GCP guidelines, complying with whichever of the requirements is longer. Opus will notify the Investigator when documents should be returned.

14.4 Records Retention

The Investigator’s site and clinical laboratory will retain all records related to the study in compliance with ICH GCP guidelines.

14.5 Amendments to the Protocol

Modifications of the signed protocol are only possible by approved protocol amendments and with the agreement of all responsible persons. The procedure for approval of a protocol amendment is identical to that for approval of the protocol. The IRB must be informed of all protocol amendments and should be asked for its opinion as to whether a full re-evaluation of the ethical aspects of the study is necessary by the committee. This should be fully documented.

The Investigator must not implement any deviation from or change to the protocol without discussion with and agreement by Opus and prior review and documented approval/favorable opinion of the amendment from the relevant ethics committee, except where it is necessary to eliminate an immediate hazard to study subjects or where the change(s) involves only logistical or administrative aspects of the study (eg, change in Study Monitor, change of telephone number).

Protocol amendments will be submitted to the appropriate authority(ies) as required by the applicable regulatory requirement(s).

15 BIBLIOGRAPHY

1. Fan-Paul NI, Li J, Miller JS, Florakis GJ. Night vision disturbances after corneal refractive surgery. *Surv Ophthalmol*. 2002;47(6):533-546.
2. Fejer TP, Girgis R. Night myopia: implications for the young driver. *Can J Ophthalmol*. 1992;27(4):172-176.
3. Hogan TS, McDaniel DD, Bartlett JD, Hart KK, Paggiarino DA. Dose-response study of dapiprazole HCl in the reversal of mydriasis induced by 2.5% phenylephrine. *J Ocul Pharmacol Ther*. 1997;13(4):297-302. doi:10.1089/jop.1997.13.297
4. Holladay JT, Dudeja DR, Chang J. Functional vision and corneal changes after laser in situ keratomileusis determined by contrast sensitivity, glare testing, and corneal topography. *J Cataract Refract Surg*. 1999;25(5):663-669.
5. Holladay JT, Meyer A, Pitlick B. Phentolamine Mesylate Ophthalmic Solution Once Daily Reduces Pupil Diameter and Improves Night Vision Disturbances. AAO Annual Meeting, PA025. 2018.
6. Isopto® Carpine (pilocarpine hydrochloride ophthalmic solution) 1%, 2% and 4% [prescribing information]. Initial U.S. Approval: 1974. 2010.
7. Martinez CE, Applegate RA, Klyce SD, McDonald MB, Medina JP, Howland HC. Effect of pupillary dilation on corneal optical aberrations after photorefractive keratectomy. *Arch Ophthalmol*. 1998;116(8):1053-1062.
8. McDonald MB, Pitlick WH, VanDevanter DR, Meyer AR, Donnenfeld ED. Phentolamine Mesylate Treatment of Severe Night Vision Complaints. AAO Annual Meetings, PO433. 2010.
9. Monestam E, Lundqvist B. Long-time results and associations between subjective visual difficulties with car driving and objective visual function 5 years after cataract surgery. *J Cataract Refract Surg*. 2006;32(1):50-55.
10. Pepose JS, Pitlick B, Meyer A, Jaber R, Charizanis K, Slonim C, et al. Phentolamine mesylate ophthalmic solution provides long lasting pupil modulation and improves visual acuity. *Invest Ophthalmol Vis Sci*. 2020;61(7):5100.
11. Pop M, Payette Y. Risk factors for night vision complaints after LASIK for myopia. *Ophthalmology*. 2004;111(1):3-10.
12. POS Investigator's Brochure; Edition 6. Ocuphire Pharma, Inc. [now Opus Genetics, Inc.] October 28, 2022.
13. Puell MC, Palomo C, Sanchez-Ramos C, Villena C. Mesopic contrast sensitivity in the presence or absence of glare in a large driver population. *Graefes Arch Clin Exp Ophthalmol*. 2004;242(9):755-761.
14. Rosen ES. Night vision disturbance. *J Cataract Refract Surg*. 2005;31(2):247-249. doi:10.1016/j.jcrs.2004.12.036
15. Steinhauer SR, Siegle GJ, Condray R, Pless M. Sympathetic and parasympathetic innervation of pupillary dilation during sustained processing. *Int J Psychophysiol*. 2004;52(1):77-86.

16. van Alphen GW. The adrenergic receptors of the intraocular muscles of the human eye. *Invest Ophthalmol*. 1976;15(6):502-505.

17. Vuity® (pilocarpine hydrochloride ophthalmic solution) 1.25% [prescribing information]. Initial U.S. Approval: 1974. 2023.

18. Yoshitomi T, Ito Y, Inomata H. Adrenergic excitatory and cholinergic inhibitory innervations in the human iris dilator. *Exp Eye Res*. 1985;40(3):453-459.

19. Yu Y, Koss MC. Alpha(1A)-adrenoceptors mediate sympathetically evoked pupillary dilation in rats. *J Pharmacol Exp Ther*. 2002;300(2):521-525.

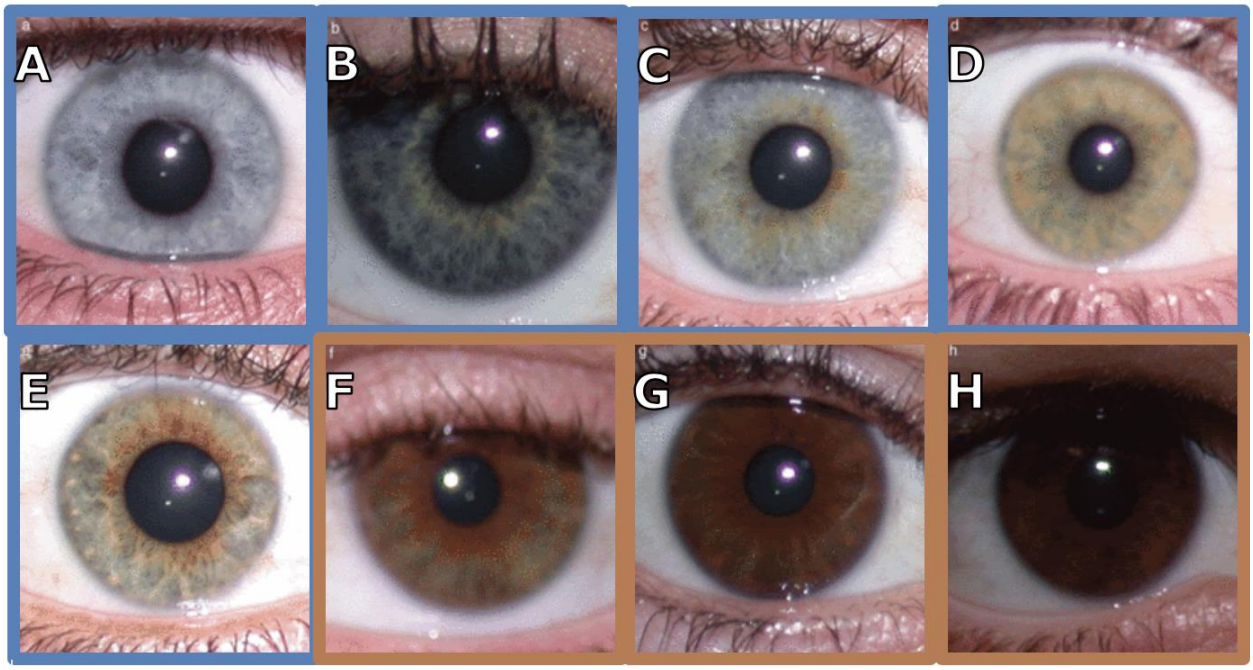
16 SUMMARY OF CHANGES

The Original Protocol (Version 1.0) was dated 01 February 2024. Protocol amendments are detailed below. **Bold text** shows additions; ~~strikethrough text~~ shows deletions. Administrative and minor edits that do not affect the content or conduct of the study may not be listed.

Protocol Amendment 1 (Version 2.0), July 11, 2025		
Section/Location	**Description of Change**	**Rationale for Change**
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

APPENDIX 1: IRIS COLOR CHART

Study enrollment includes both light and dark-colored eyes**.** Examples of light irides (pictures a, b, c, d, and e) and dark irides (pictures f, g, and h) for the purposes of this study are detailed in the chart below.



a = Light blue
b = Dark blue
c = Blue with peripupillary brown
d = Uniform green
e = Green with brown iris ring
f = Central brown and peripheral green
g = Brown with some peripheral green
h = Brown

APPENDIX 2: SUBJECT QUESTIONNAIRE

Vision and Night Driving Questionnaire (VND-Q)

The Vision and Night Driving Questionnaire (VND-Q) is a 9-item self-report measure of vision-related night driving difficulties that uses a 5-point Likert-type scale format, with responses ranging from 1 = 'No difficulty' to 5 = 'Extreme difficulty'.

The VND-Q authors, Kimlin et al., grant permission to use the questionnaire by citing the following reference:

Kimlin JA, Black A, Djaja N, Wood JM. (2016) Development and validation of a vision and night driving questionnaire. Ophthalmic and Physiological Optics, 36(4), 465-476.

Changes to the VND-Q may be made without the written permission from the authors. However, all such changes should be clearly identified as having been made by the recipient.

Instructions on use

The VND-Q has been designed and validated for use as a self-administered questionnaire.

Vision and Night Driving Questionnaire (VND-Q)

This questionnaire asks about your visual difficulties when driving at night. For each question, please select the answer that best describes your situation and answer **as if you were wearing the glasses or contact lenses that you would normally wear (if any) for night driving**. Please answer each question as accurately and as quickly as possible. Do not dwell too long on any particular question.

How much difficulty do you, or would you have, with the following night driving tasks?

1. Seeing dark coloured cars when driving at night?	No difficulty ..... 1 A little difficulty ..... 2 Moderate difficulty ..... 3 A lot of difficulty ..... 4 Extreme difficulty 5
2. Seeing pedestrians or animals on the road side when driving at night?	No difficulty ..... 1 A little difficulty ..... 2 Moderate difficulty ..... 3 A lot of difficulty ..... 4 Extreme difficulty 5
3. Reading street signs when driving at night?	No difficulty ..... 1 A little difficulty ..... 2 Moderate difficulty ..... 3 A lot of difficulty ..... 4 Extreme difficulty 5
4. Seeing the road because of oncoming headlights when driving at night?	No difficulty ..... 1 A little difficulty ..... 2 Moderate difficulty ..... 3 A lot of difficulty ..... 4 Extreme difficulty 5

5. Seeing because of glare when driving at dawn or dusk?	No difficulty ..... 1 A little difficulty ..... 2 Moderate difficulty..... 3 A lot of difficulty..... 4 Extreme difficulty..... 5
6. Adjusting after passing headlights from oncoming cars when driving at night?	No difficulty ..... 1 A little difficulty ..... 2 Moderate difficulty..... 3 A lot of difficulty..... 4 Extreme difficulty..... 5
7. Judging the distance to your turn-off or exit while driving at night?	No difficulty ..... 1 A little difficulty ..... 2 Moderate difficulty..... 3 A lot of difficulty..... 4 Extreme difficulty..... 5
8. Judging the distance between you and other moving cars when driving at night?	No difficulty ..... 1 A little difficulty ..... 2 Moderate difficulty..... 3 A lot of difficulty..... 4 Extreme difficulty..... 5
9. Seeing the road in rain or poor weather when driving at night?	No difficulty ..... 1 A little difficulty ..... 2 Moderate difficulty..... 3 A lot of difficulty..... 4 Extreme difficulty..... 5

Vision and Night Driving Questionnaire (VND-Q) – Ordinal to Interval Conversion Table

The ordinal VND-Q score, by summing the items response scores, can be converted to a Rasch-scaled interval-level score ranging from 0 to 10, thus making the VND-Q score suitable for parametric statistics. Higher scores represent more vision-related night driving difficulties. This table cannot be used for respondents with missing data.

Ordinal Score	Logits	Interval Scale Score
9	-7.72	0.00
10	-6.38	0.88
11	-5.49	1.46
12	-4.87	1.87
13	-4.35	2.21
14	-3.9	2.50
15	-3.49	2.77
16	-3.1	3.03
17	-2.74	3.26
18	-2.4	3.48
19	-2.08	3.69
20	-1.77	3.90
21	-1.48	4.09
22	-1.2	4.27
23	-0.93	4.45
24	-0.67	4.62
25	-0.41	4.79
26	-0.16	4.95
27	0.09	5.11
28	0.34	5.28
29	0.59	5.44
30	0.84	5.61

Ordinal Score	Logits	Interval Scale Score
31	1.1	5.78
32	1.35	5.94
33	1.61	6.11
34	1.87	6.28
35	2.15	6.46
36	2.43	6.65
37	2.72	6.84
38	3.04	7.05
39	3.37	7.26
40	3.74	7.50
41	4.16	7.78
42	4.64	8.09
43	5.26	8.50
44	6.18	9.10
45	7.55	10.00

APPENDIX 3: ADRENERGIC AND CHOLINERGIC DRUGS

The following drugs are examples of drugs which cannot be used within 7 days prior to Screening or during the study unless the drug, dose, and regimen has been consistent for the 7 days prior to Screening; ***however, tamsulosin is specifically excluded***. *This list is not inclusive of all drugs in these classes. If there is any doubt, please consult with the Medical Monitor.*

Alpha-1-agonists
- Methyl norepinephrine
- Naphazoline
- Oxymetazoline
- Tetrahydrozoline
- Phenylephrine
- Xylometazoline
- Metaraminol
- Methoxamine
- Midodrine
- Amidephrine

Alpha-2-agonists
- Brimonidine
- Clonidine
- Guanfacine
- Guanabenz
- Guanoxabenz
- Guanethidine
- Xylazine
- Tizanidine
- Methyldopa
- Norepinephrine
- Lofexidine
- Medetomidine

Undetermined alpha agonists
- Ergotamine
- Etilefrine
- Indanidine
- Mephentermine
- Metaraminol
- Methoxamine

Non-selective alpha-antagonists
- Phenoxybenzamine
- Tolazoline
- Labetalol
- Carvedilol
- Trazodone
- Phentolamine

Alpha-1-antagonists
- Alfuzosin
- Prazosin
- Doxazosin
- Tamsulosin
- Terazosin
- Silodosin
- Fuzosin

Alpha-2-antagonists
- Atipamezole
- Idazoxan
- Yohimbine
- Raulscine
- Mirtazapine

Direct-acting acetylcholine receptor agonists

Choline esters
- Acetylcholine
- Bethanechol
- Carbachol
- Methacholine

Plant alkaloids
- Arecoline
- Nicotine
- Muscarine
- Pilocarpine

Indirect-acting acetylcholine receptor agonists

Reversible cholinesterase inhibitors
- Donepezil
- Edrophonium
- Neostigmine
- Physostigmine
- Pyridostigmine
- Rivastigmine
- Tacrine
- Caffeine
- Huperzine A

Irreversible cholinesterase inhibitors
- Echothiophate
- Isoflurophate
- Malathion

Acetylcholine receptor antagonists
- Scopolamine
- Dicycloverine

Gastrointestinal
- Atropine
- Belladonna

Parkinsonism
- Amantadine
- Benztropine
- Biperiden
- Trihexyphenidyl

Antimuscarinic agents

Antipsychotics (clozapine, quetiapine)
- Atropine
- Benztropine
- Biperiden
- Chlorpheniramine

Certain SSRIs (Paroxetine)
- Dicyclomine (Dicycloverine)
- Dimenhydrinate
- Diphenhydramine
- Doxepin
- Doxylamine
- Flavoxate
- Glycopyrrolate
- Glycopyrronium
- Hyoscyamine
- Ipratropium
- Orphenadrine
- Oxitropium
- Oxybutynin
- Promethazine
- Propantheline bromide
- Scopolamine

		Tolterodine Oxybutynin Ipratropium Mamba Toxin (MT_7) Pirenzepine Telenzepine *Antivertigo* Meclizine Scopolamine	Solifenacin Tolterodine Tiotropium *Tricyclic antidepressants* Trihexyphenidyl Tropicamide Umeclidinium *Antinicotinic agents* Bupropion Dextromethorphan Doxacurium Hexamethonium Mecamylamine Tubocurarine