CONFIDENTIAL

Clinical Study Phase 2 Protocol
OPI-NYXP-201
VEGA-1

Randomized, Placebo-Controlled, Double-Masked Study of the Safety and Efficacy of Nyxol (0.75% Phentolamine Ophthalmic Solution) with Low-Dose (0.4%) Pilocarpine Eye Drops in Subjects with Presbyopia

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Version: **01**
Original: **November 30, 2020**
Amendment 1: **n/a**

SPONSOR SIGNATURE & CONTACTS

Study Title:	Randomized, Placebo-Controlled, Double-Masked Study of the Safety and Efficacy of Nyxol (0.75% Phentolamine Ophthalmic Solution) with Low-Dose (0.4%) Pilocarpine Eye Drops in Subjects with Presbyopia
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Person authorized to sign the protocol and protocol amendment(s) for the sponsor, Ocuphire Pharma, Inc.

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30 Nov 2020

Date

INVESTIGATOR'S AGREEMENT

OPI-NYXP-201
VEGA-1

Randomized, Placebo-Controlled, Double-Masked Study of the Safety and Efficacy of Nyxol (0.75% Phentolamine Ophthalmic Solution) with Low-Dose (0.4%) Pilocarpine Eye Drops in Subjects with Presbyopia

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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 the protection of human subjects (21 CFR 50), 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
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ABBREVIATIONS AND TERMS

Abbreviation	*Full term*
AE	adverse event
ANCOVA	analysis of covariance
ARP	All Randomized Population
BCDVA	best-corrected distance visual acuity
BCIVA	best-corrected intermediate visual acuity
BP	blood pressure
°C	degrees Celsius
CCLRU	Cornea and Contact Lens Research Unit
CFR	Code of Federal Regulations
CI	confidence interval
CRA	clinical research associate
CRO	clinical research organization
DCNVA	distance-corrected near visual acuity
DLD	dim light vision disturbances (also referred to as night vision disturbances or NVD)
eCRF	electronic Case Report Form
ETDRS	Early Treatment Diabetic Retinopathy Study
°F	degrees Fahrenheit
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HR	heart rate
IB	Investigators' Brochure
ICH	International Council for Harmonisation
IND	Investigational New Drug
IOP	intraocular pressure
IRB	Institutional Review Board
IUD	intrauterine device

LCVA	low-contrast visual acuity
LDP	Low-Dose (0.4%) Pilocarpine Ophthalmic Solution
LDPE	low-density polyethylene
LSM	least-squares mean
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intention-to-Treat
NVD	night vision disturbances
Nyxol	0.75% Phentolamine Ophthalmic Solution or 1% Phentolamine Mesylate Ophthalmic Solution
Nyxol + LDP	Nyxol dosed in the evening with LDP dosed during the day
OD	oculus dexter (right eye)
OR	odds ratio
OTC	over-the-counter
OU	oculus uterque (both eyes)
PD	pupil diameter
POS	Phentolamine Ophthalmic Solution
PP	Per Protocol
SAE	serious adverse event
SOC	system organ class
SNV	severe night vision disturbances
SP	Safety Population
TEAE	treatment-emergent adverse event
US	United States
USP	United States Pharmacopeia
VA	visual acuity
WHODrug	World Health Organization Drug Dictionary

1. STUDY SUMMARY

Study Number	**OPI-NYXP-201**
Clinical Phase	Phase 2
Type of Study	Randomized, placebo-controlled, double-masked study of the safety and efficacy of Nyxol® Eye Drops (0.75% Phentolamine Ophthalmic Solution [POS]) with Low-Dose (0.4%) Pilocarpine Ophthalmic Solution (LDP) in subjects with presbyopia
Name of Investigational Product	Nyxol Eye Drops – 0.75% POS (Treatment 1) 0.4% LDP (Treatment 2)
Duration of Study	Approximately 5 to 9 days, including Visit 1 (Screening/Baseline), Visit 2 (Treatment), and Visit 3 (Safety Follow-Up Call)
Rationale	Presbyopia is an age-related condition typically starting at around 40 years of age. It is caused by the inability of the aging lens to dynamically change shape, curvature, and dioptric power in an effort to focus images of nearby objects onto the retina. Presbyopia has a significantly negative impact on quality of life, interfering with reading, use of computers or hand-held devices, and seeing the dashboard of a car. There are no current pharmacological therapies approved, but there is some evidence that decreasing pupil diameter (PD), especially to a size of 1.6 to 2 mm to create a "pinhole" effect, can improve visual acuity (VA) by increasing the depth of focus. The combination of Nyxol (0.75% POS) and 0.4% LDP (Nyxol + LDP) could be a potential treatment option for presbyopia. Specifically, Nyxol would be dosed once daily at or near bedtime and LDP dosed in the morning. Nyxol + LDP dosing can potentially reduce PD to ≤ 2.0 mm, which has the potential to improve near VA due to a "pinhole" effect. Phentolamine is a non-selective alpha-1 and alpha-2 adrenergic receptor antagonist that inhibits contraction of the iris dilator muscle, resulting in a smaller pupil size. Pilocarpine hydrochloride is a cholinergic, parasympathomimetic agent which acts through stimulation of muscarinic receptors on smooth muscles such as the iris and secretory glands. Pilocarpine reduces pupil size through stimulating contraction of the iris sphincter muscle. In a Phase 2 trial, a statistically significant number of presbyopic subjects treated with Nyxol demonstrated ≥ 1 line of improvement

	in distance-corrected near visual acuity (DCNVA) compared to Placebo-treated subjects, with a trend for a 2-line improvement at all timepoints. In these studies, Nyxol was generally well tolerated in the eye (most common complaint was mild-to-moderate ocular hyperemia) and there were no clinically meaningful systemic effects, as measured by heart rate (HR) and blood pressure (BP).
Study Objectives	The objectives of this study are: • To evaluate the efficacy of Nyxol + LDP to improve DCNVA compared to Placebo alone in subjects with presbyopia • To evaluate the efficacy of Nyxol + LDP to prevent < 1 line of loss of BCDVA • To evaluate the efficacy of Nyxol + LDP to improve DCNVA compared to Nyxol alone or LDP alone • To evaluate the ability of Nyxol + LDP to produce the "pinhole" pupil size (approximately 1.6-2 mm) • To evaluate the efficacy of Nyxol + LDP to improve BCIVA and BCDVA • To evaluate the effect of iris color on the efficacy of Nyxol + LDP • To evaluate the ocular and systemic safety of Nyxol + LDP and each component individually The Sponsor intends to use this Phase 2 study to evaluate Nyxol + LDP for the chronic indication of "temporary treatment of presbyopia."
Design	This is a placebo-controlled, double-masked, multiple-dose, Phase 2 study in approximately 152 randomized subjects with presbyopia (approximately 140 evaluable for efficacy), evaluated for safety and efficacy following administration of masked Nyxol or Placebo (Treatment 1) at or near bedtime for 3 to 4 days, with administration of LDP or No Treatment (Treatment 2) at the next visit (Visit 2) in the morning. Measurements will be made at multiple visits and timepoints, and analysis will consist of comparison across 4 treatment arms: • Nyxol + LDP • Nyxol + No Treatment 2 (Nyxol alone) • Placebo + LDP • Placebo + No Treatment 2 (Placebo alone) Subjects will be randomized 4:3:3:4 into the above groups. Randomization will be stratified 1:1 by iris color.

	After randomization, masked Treatment 1 study medications (Nyxol or Placebo) will be dispensed. Treatment 1 study medication will be taken by the subject at or near bedtime (approximately 8PM-10PM). The treatment will start the night of Visit 1 (Screening/Baseline) or at the appropriate evening in order for Treatment 1 to be taken daily for 3 to 4 consecutive days immediately prior to Visit 2. Treatment 2 (LDP or No Treatment) is unmasked and will be administered at Visit 2 by a designated, unmasked, Site Staff member, distinct from the Site Staff member recording measurements/assessments. ***Treatment 1 (Nyxol or Placebo) will be administered to both eyes (OU) by the subject.*** ***Treatment 2 (LDP or No Treatment) will be administered OU by the Site Staff.*** The study eye is defined as the eye with worse Baseline DCNVA. In the case where both eyes have the same Baseline DCNVA, the study eye will be the right eye (OD). The non-study eye will be the fellow eye. The study eye and fellow eye will both be evaluated at all assessments.
Subject Population	Approximately 152 randomized subjects with presbyopia (approximately 140 evaluable for efficacy)
Inclusion Criteria	1. Males or females ≥ 40 and ≤ 64 years of age. 2. Able to comply with all protocol-mandated procedures independently and to attend all scheduled office visits. 3. Able and willing to give signed informed consent. 4. Able to self-administer study medication throughout the study period. 5. BCDVA of 0.0 LogMAR (20/20 Snellen equivalent) or better in each eye under photopic conditions. 6. DCNVA of 0.4 LogMAR (20/50 Snellen equivalent) or worse under photopic conditions in each eye and binocularly. 7. Subjects who depend on reading glasses or bifocals in which their binocular best-corrected near VA is 0.1 LogMAR (20/25 Snellen equivalent) or better.
Exclusion Criteria	**Ophthalmic (in either eye):** 1. Use of any topical prescription or over-the-counter (OTC) ophthalmic medications of any kind within 7 days of Screening until study completion, with the exception of lid scrubs with

	OTC products (e.g., OCuSOFT® lid scrub, SteriLid®, baby shampoo, etc.). 2. Use of any OTC artificial tears (preserved or unpreserved) at least once per day within 7 days of Screening until study completion. 3. Current use of any topical ophthalmic therapy for dry eye (e.g., Restasis, Xiidra, etc.). 4. Tear break-up time of < 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). 5. Clinically significant ocular disease (e.g., cataract, glaucoma, corneal edema, uveitis, retinal degeneration, loss of visual field, any macular pathology) that might interfere with the study as deemed by the Investigator. 6. Recent or current evidence of ocular infection or inflammation in either eye (such as current evidence of clinically significant blepharitis, conjunctivitis, keratitis, etc.). Subjects must be symptom free for at least 7 days. 7. Any history of herpes simplex or herpes zoster keratitis. 8. History of diabetic retinopathy or diabetic macular edema. 9. Known allergy, hypersensitivity, or contraindication to any component of the phentolamine, pilocarpine, or vehicle formulations. 10. History of cauterization of the punctum or punctal plug (silicone or collagen) insertion or removal. 11. Ocular trauma, ocular surgery (e.g., intraocular lenses), ocular laser treatment within the 6 months prior to Screening. Any subject with multifocal intraocular lenses are excluded. 12. History of any traumatic (surgical or nonsurgical) or non-traumatic condition affecting the pupil or iris (e.g., irregularly shaped pupil, neurogenic pupil disorder, iris atrophy, iridotomy, iridectomy, iritis, etc.). 13. Unwilling or unable to discontinue use of contact lenses at Screening until study completion. 14. Conjunctival hyperemia ≥ grade 2 on the Cornea and Contact Lens Research Unit (CCLRU) 4-point scale. **Systemic:** 15. Known hypersensitivity or contraindication to alpha- and/or beta-adrenoceptor antagonists (e.g., chronic obstructive pulmonary disease or bronchial asthma; abnormally low BP or

	HR; second- or third-degree heart blockage or congestive heart failure). 16. Known hypersensitivity or contraindication to any systemic cholinergic parasympathomimetic agents. 17. Clinically significant systemic disease (e.g., uncontrolled diabetes, myasthenia gravis, cancer, hepatic, renal, endocrine, or cardiovascular disorders) that might interfere with the study as deemed by the Investigator. 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. 20. Females of childbearing potential who are pregnant, nursing, planning a pregnancy, or not using a medically acceptable form of birth control. Acceptable methods include the use of at least one of the following: intrauterine device (IUD), 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 with post-tubal ligation must have a negative urine pregnancy test result at Visit 1 (Screening/Baseline). 21. Resting HR outside the specified range of 50 to 110 beats per minute following at least a 5-minute rest period in the sitting position at the Screening Visit 1. HR may be repeated **only once** if outside the specified range, following another 5-minute rest period in the sitting position. 22. Hypertension with resting diastolic BP > 105 mmHg or systolic BP > 160 mmHg following at least a 5-minute rest period in the sitting position at the Screening Visit 1. BP may be repeated **only once** if outside the specified range, following another 5-minute rest period in the sitting position.
Screening/Baseline Visit (Visit 1)	Individuals who are potential subjects are identified by the study center to schedule the Screening Visit. The Screening Visit shall occur the same day as the Baseline Visit. Once a subject arrives at the study center, a member of the Site Staff will interview the individual as to their qualifications for participation in the study, and if the subject wishes to continue, the informed consent form is signed, and a subject number is assigned.

	The start of Screening includes an explanation of the study, a medical and ophthalmic history, and demographics. [redacted] This shall be followed by a urine pregnancy test for females of childbearing potential (Exclusion Criterion #20), and HR/BP (Exclusion Criteria #21-22). The subject will then undergo several VA measurements, including photopic BCDVA as well as [redacted] DCNVA ([redacted] In addition, subjects will undergo measurement of photopic best-corrected intermediate visual acuity (BCIVA) and mesopic BCDVA. The screening assessment will also include an ophthalmic examination that includes assessment of PD, biomicroscopy, dry eye examination with tear break-up time testing and corneal fluorescein staining ([redacted]), intraocular pressure (IOP) measurement (using a Tono-Pen), and direct or indirect ophthalmoscopy without dilation. In addition, conjunctival hyperemia will be assessed visually with a 4-point grading scale using images from the CCLRU [redacted]). If all eligibility criteria are met, the subject will be randomized into the study. After randomization, masked Treatment 1 study medications (Nyxol or Placebo) will be dispensed. All treatments and assessments will be administered OU.
Visit 2 Treatment + Assessments	On the morning of Visit 2, subjects will undergo certain assessments immediately *prior* to receiving Treatment 2 at 0 minutes: • Urine pregnancy test for females of childbearing potential • HR/BP • Concomitant medications • PD • DCNVA ([redacted]), BCDVA ([redacted]), BCIVA [redacted]) – [redacted] Treatment 2 (LDP or No Treatment) is unmasked and will be administered on Visit 2 by a designated, unmasked, Site Staff member, distinct from the Site Staff member recording measurements/assessments.

	After instillation of Treatment 2 (LDP or No Treatment), subjects will undergo certain assessments at multiple timepoints after Time 0 (from 30 minutes to 6 hours): • PD • DCNVA ([redacted]), BCDVA ([redacted]), BCIVA [redacted]) – [redacted] • Conjunctival hyperemia • Adverse events (AEs) • Subjective ocular tolerability Biomicroscopy, IOP, and ophthalmoscopy will be performed at the 6-hour timepoint.
Follow-Up Call (Visit 3)	Safety Follow-Up Call (Visit 3; 1-3 days after Treatment Visit 2) subject assessments: • Concomitant medications • AEs
Number of Investigational Sites	Approximately 20 sites
Estimated Total Sample Size	Approximately 152 randomized subjects, with approximately 140 subjects evaluable for efficacy
Sample Size Justification	A sample size of approximately 140 subjects (40 for Nyxol + LDP and Placebo alone arms and 30 for each of the other 2 arms) who are evaluable for efficacy is needed for the study. The primary treatment comparison will be Nyxol + LDP compared to Placebo alone. Using $\alpha = 0.05$ significance and a two-tailed test, 76 total subjects (38 subjects each in the Nyxol + LDP and Placebo alone arms) [redacted] Subjects will be randomized 4:3:3:4 to Nyxol + LDP, Nyxol + No Treatment 2 (Nyxol alone), Placebo + LDP, and Placebo + No Treatment 2 (Placebo alone), respectively. It is assumed that there will be approximately 7% drop-out between Visit 1 and end of Visit 2. To account for this drop-out, a total of approximately 152 subjects will be randomized into the study.

Primary Efficacy Endpoint	The primary efficacy endpoint is the percent of subjects with ≥ 15 letters of improvement in photopic binocular DCNVA on Visit 2 at 1 hour with Nyxol + LDP compared to Placebo alone. The improvement in binocular DCNVA for each subject is relative to the subject's own Baseline value (Visit 1).
Secondary Efficacy Endpoints	Secondary efficacy endpoints (for the study eye, the fellow eye, and binocular) will include: [redacted]

	[redacted] [redacted] [redacted] [redacted] [redacted] [redacted] [redacted] [redacted] [redacted], Placebo + [redacted] [redacted]

	[redacted] Measurements: • DCNVA Reading/Near will be measured under [redacted] • BCDVA will be measured under photopic conditions by a high-contrast Standard Early Treatment Diabetic Retinopathy Study (ETDRS) i[redacted] • BCIVA will be measured under photopic conditions by a [redacted] • PD will be measured with a [redacted] All of the efficacy endpoints will be analyzed overall and by [redacted] at all timepoints. Subjects will also be analyzed by study eye, fellow eye, and binocular. All of the efficacy endpoints will also be analyzed by Modified Intention-to-Treat (mITT) and Per Protocol (PP) populations.
Safety Endpoints	The primary safety measures are conjunctival hyperemia, subjective ocular tolerability (Visit 2), and AEs. Analysis for conjunctival hyperemia includes change from Baseline (Visit 1) in the conjunctival hyperemia grading scale (CCLRU images) at each visit, for study eye and fellow eye. Any increase of 2 units in conjunctival hyperemia between consecutive visits is considered an AE. Other safety measures include IOP, biomicroscopy, ophthalmoscopy, and systemic safety as measured by HR and BP. Urine pregnancy tests for females of childbearing potential will be conducted. Measurements: • Conjunctival hyperemia will be assessed visually with a grading scale (0-3) using images from the CCLRU • Subjective ocular tolerability will be measured on a 4-point scale (0-3) • IOP will be measured with the Tono-Pen

Study Medications, Dose and Mode of Administration	***Treatment 1 (Masked)*** Nyxol® Eye Drops (0.75% Phentolamine Ophthalmic Solution): Treatment 1 study medication will be taken by the subject at or near bedtime (approximately 8PM-10PM) starting the night of Visit 1 (Screening/Baseline) or at the appropriate evening in order for Treatment 1 to be taken daily for 3 to 4 consecutive days immediately prior to Visit 2. Placebo (Nyxol vehicle): One drop of Placebo will be administered in each eye daily at or near bedtime (approximately 8PM-10PM) starting the night of Visit 1 (Screening/Baseline) or at the appropriate evening in order for Treatment 1 to be taken daily for 3 to 4 consecutive days immediately prior to Visit 2. Subjects will be given enough Nyxol or Placebo for 4 doses. ***Treatment 2 (Unmasked)*** LDP (Low-Dose [0.4%] Pilocarpine Ophthalmic Solution): One drop of LDP will be administered in each eye by Site Staff at Time 0 minutes during Visit 2. No Treatment: No drop of LDP will be administered by Site Staff at Time 0 minutes during Visit 2.
Duration of Subject Participation and Study	The total length of subject participation is approximately 5 to 9 days (accommodating for weekends) with 2 clinic visits and one telephone call follow-up as summarized below: • Screening/Baseline Visit 1 (Day 1) • Treatment Visit 2 (Day 4 to 6) • Follow-up Call Visit 3 (Day 5 to 9) The execution of the entire study (first subject screen through last randomized subject completed) is expected to take approximately 6 to 9 months.

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2. INTRODUCTION

2.1. Investigational Products

The combination of Nyxol (0.75% Phentolamine Ophthalmic Solution [POS]) and Low-Dose (0.4%) Pilocarpine Ophthalmic Solution (LDP) is to be studied for the indication of "temporary treatment of presbyopia." Specifically, Nyxol would be dosed once daily at or near bedtime and LDP dosed in the morning. Nyxol + LDP dosing can potentially reduce pupil diameter (PD) size to ≤ 2.0 mm, which has the potential to improve near visual acuity (VA) due to a "pinhole" effect. In this trial, Nyxol and Placebo are referred to as Treatment 1, and LDP and No Treatment are referred to as Treatment 2.

Treatment 1 (Masked)

The test product is Nyxol® Eye Drops – 0.75% Phentolamine Ophthalmic Solution (Nyxol), a non-selective alpha-1 and alpha-2 adrenergic receptor antagonist that inhibits contraction of the iris dilator muscle, resulting in a smaller pupil size. Note that the concentration of 0.75% refers to phentolamine free base and is the same—and used in place of—1% phentolamine mesylate drug substance, which was how Nyxol had been described in all prior studies.

Placebo control is Nyxol vehicle alone.

One drop of Nyxol or Placebo will be administered in each eye daily at or near bedtime (approximately 8PM-10PM) starting the night of Visit 1 (Screening/Baseline) or at the appropriate evening in order for Treatment 1 to be taken daily for 3 to 4 consecutive days immediately prior to Visit 2.

Subjects will be given enough Nyxol or Placebo for 4 doses.

Treatment 2 (Unmasked)

Low-Dose (0.4%) Pilocarpine Ophthalmic Solution (LDP) is a direct-acting cholinergic, parasympathomimetic agent which acts through direct stimulation of muscarinic receptors and smooth muscle such as the iris and secretory glands. Pilocarpine reduces pupil size through stimulating contraction of the iris sphincter muscle.

For those who receive LDP, one drop of LDP will be administered in each eye by Site Staff at Time 0 minutes during Visit 2.

For those who receive No Treatment, no drop of LDP will be administered by Site Staff at Time 0 minutes during Visit 2.

2.2. Findings From Nonclinical and Clinical Studies

Nyxol

Detailed findings from nonclinical and clinical studies and potential risk are provided in the Investigators' Brochure (IB) (2020).

Nyxol has been assessed in 7 Investigator-initiated and sponsored Phase 1 and Phase 2 clinical trials. Across all trials, 168 of 232 adult subjects were exposed to at least one dose of Phentolamine Mesylate Ophthalmic Solution. The data presented below are from Nyxol alone.

In prior clinical trials, Nyxol has demonstrated a consistent ability to decrease PD by approximately 20% (~1-1.5 mm) in both mesopic and photopic conditions. Nyxol has been found to be efficacious for more than 24 hours with an onset of action of approximately 30 to 60 minutes. Key PD data are summarized below (Table 1).

Table 1: Efficacy of 1% Nyxol in Reducing Pupil Diameter in Mesopic Conditions in Phase 2 Trials

Study	Group	Mesopic Conditions				
		Pre-Treatment (Baseline) Pupil Diameter	**Post-Treatment Pupil Diameter**	**Change (%)**	**p-value compared to baseline**	**p-value compared to placebo**
NYX-SNV (1)	Placebo (N = 16)	6.6 mm	6.4 mm	-0.2 mm (-3%)	p = 0.08	**p < 0.0001**
	1.0% Nyxol (N = 32)	6.5 mm	5.2 mm	**-1.3 mm (-20%)**	**p < 0.0001**	
NYX-01a2 (2)	Placebo (N = 38)	6.25 mm	6.31 mm	0.07 mm (+1%)	p = 0.6	**p < 0.0001**
	1.0% Nyxol (N = 40)	6.17 mm	5.31 mm	**-0.86 mm (-14%)**	**p < 0.0001**	
NYXG-201 (3)	Placebo (N = 20)	4.57 mm	4.52 mm	-0.05 mm (-1%)	p = 0.6178	**p < 0.0001**
	1.0% Nyxol (N = 19)	4.69 mm	3.70 mm	**-1.00 mm (-21%)**	**p < 0.0001**	

Nyxol was observed to be well tolerated at single doses up to and including 1.0% daily in each eye. This includes 59 subjects who received multiple doses of up to 1% Nyxol for at least 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 healthy volunteers or subjects reported a treatment-emergent serious adverse event (SAE). 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). AEs reported were mild to moderate in intensity with the most common being transient conjunctival hyperemia and ocular irritation; however, Nyxol dosing at or near bedtime was observed to mitigate or minimize these side effects during the daytime.

Phase 2 Trial in a Glaucomatous and Presbyopic Population (ORION-1, NYXG-201):

ORION-1 (NYXG-201) was a double-masked, randomized, placebo-controlled, multi-center trial of 1% Nyxol compared with placebo ophthalmic solution for 14 days in subjects with open-angle glaucoma or ocular hypertension. After screening was performed based on inclusion and exclusion criteria, a total of 39 elderly subjects (median age of 63) were randomized into the trial (Nyxol arm, n = 19; placebo arm, n = 20). These subjects were either treatment-naïve or were previously taking intraocular pressure (IOP)-lowering medication and were washed out for 30 days prior to dosing. Subjects took their study medication (Nyxol or placebo) in both eyes (OU) between 8PM and 10PM every evening for 14 days. Assessments were made on Day 1, Day 8, Day 15, and Day 16. The primary efficacy endpoint was change from baseline in mean diurnal IOP at Day 15. Mean diurnal IOP is the mean of the IOP measurements at 3 timepoints (8AM, 10AM, 4PM). Secondary efficacy endpoints included change in PD, change in distance-corrected near visual acuity (DCNVA), and change in best-corrected distance visual acuity (BCDVA), as well as additional IOP analyses. Safety assessments included measurements of conjunctival hyperemia (using the Cornea and Contact Lens Research Unit [CCLRU] grading 4-point scale [0-3]), AEs, HR, BP, concomitant medications, and pregnancy.

In summary, Nyxol had statistically significant reductions in PD under photopic and mesopic conditions that were sustained for 36 hours post-dosing (Table 1). A statistically significant number of subjects treated with Nyxol compared to placebo demonstrated ≥ 1 line of improvement in DCNVA (63.2% with Nyxol vs 20.0% with placebo), with a trend for a 2-line improvement at all timepoints. There was no statistical difference in conjunctival hyperemia

compared to placebo. In summary, daily evening dosing of Nyxol was found to be well tolerated with no daytime conjunctival redness and demonstrated improvement in DCNVA with sustained PD reduction in a glaucomatous and presbyopic population. Smaller pupil size can have beneficial effects in improving near VA in presbyopic subjects, which is relevant to the VEGA-1 trial.

Regarding safety, Nyxol 1% was well tolerated and there were no major ocular or systemic safety issues. An evening dose regimen minimized eye redness during the daytime while benefiting near VA in an elderly population. The incidence of treatment-emergent adverse events (TEAEs) was higher in the Nyxol arm compared with the placebo arm (31.6% versus 5.0%) but all TEAEs were mild in severity, with no serious TEAEs or TEAEs leading to withdrawal or study medication discontinuation. Most TEAEs were considered related to study medication, with the most frequent being conjunctival hyperemia and burning on instillation (each seen in 16% of subjects treated with Nyxol). Although conjunctival redness scores increased in the Nyxol arm at Day 8, Day 15, and Day 16, the scores in the Nyxol arm at any post-baseline timepoint did not demonstrate a statistically significant difference from scores in the placebo arm. Mean systolic and diastolic BPs and HRs were relatively unchanged and remained within normal range throughout the duration of the trial and were similar between arms. Neither biomicroscopic nor ophthalmoscopic examination showed any clinically significant abnormalities at Screening or at Day 15. There was no worsening of distance VA, near VA, or IOP.

Phase 2 Trial in Patients With Severe NVD (NYX-SNV):

NYX-SNV was a double-masked, randomized, placebo-controlled, single-dose trial assessing the tolerability and effect of a single topical drop of 1.0% solution of phentolamine mesylate in Tears Naturale II in each eye or Tears Naturale II (placebo) on PD, contrast sensitivity (CS), VA, and wavefront aberrometry. A total of 24 patients (median age of 39) with severe night vision (SNV) complaints were randomly assigned 2:1 to treatment groups (active treatment, n = 16; placebo control, n = 8). Each group was treated with one drop of test article in each eye. The primary endpoint was a statistically significant improvement in the mean change in monocular CS scores under mesopic conditions at each of 5 spatial frequencies. Key secondary endpoints included measurements of low-contrast visual acuity (LCVA) under mesopic and photopic conditions, change in PD, and percent of subjects with an improvement in CS (at multiple frequencies), which were recorded at baseline (prior to treatment administration) and approximately 2 hours after administration.

In summary, in NYX-SNV, 1% Nyxol demonstrated statistically significant reductions in PD and improvement in LCVA under photopic and mesopic lighting conditions, as well as individual CS frequency improvements. Treatment with 1% Nyxol further exhibited a statistically significant reduction in aberration errors (errors that affect light transmission in specific PD sizes). Additionally, mean PD decreased an average of 1.3 mm ($p < 0.0001$), or ~20%, for phentolamine mesylate–treated patients, whereas mean PD of placebo-treated patients did not significantly change between pre-treatment and post-treatment. The difference in mean PD change between treatment groups was also statistically significant (1.1 mm; $p < 0.0001$) (Table 1).

No SAEs or other AEs were reported during the trial. Overall, study treatment appeared to be well tolerated. No meaningful differences in mean HR or mean systolic and diastolic BP between treatment groups were observed. Treatment with phentolamine mesylate caused a statistically significant elevation in mean change from baseline in conjunctival hyperemia between the 2 treatment groups (+38.6 mm versus +12.1 mm for placebo; $p < 0.0004$; range, 0 mm [no redness]

– 100 mm [maximal redness]). The mean change in IOP of phentolamine mesylate–treated eyes from screening to 2 to 3 hours post-treatment (-1.8 mmHg) was statistically significant ($p < 0.0004$).

It is important to note that similar results were found in NYX-01a2, which was a 15-day, double-masked, randomized, placebo-controlled trial in patients with severe dim light vision disturbances (DLD). The NYX-01a2 trial demonstrated a dose response favoring 1% Nyxol. Further, statistically significant reductions in PD, trends in improvement in LCVA under bright and dim lighting conditions were shown. Durability of effect on PD was observed 24 hours later for Nyxol with daily morning doses.

Pilocarpine

A detailed report of pilocarpine, its product label, properties, and potential risk are provided in the IB (2020).

Pilocarpine hydrochloride has not been studied in prior clinical trials by Ocuphire. However, there is extensive literature on its ability, at various concentrations, to reduce PD. These pupil-constricting effects are counteracted by a dose-dependent side effect profile, notably browache and stinging in the eye upon topical application of the drug.

In an effort to better characterize an efficacious dose of pilocarpine that would have minimal side effects, Ocuphire conducted an expansive review of the medical literature concerning experiments of various doses of pilocarpine and their effects on PD and side effect profile. Leavitt et al. found that normal pupils constrict to dilute concentrations of 0.25% or 0.125% pilocarpine (1.7 and 0.6 mm, respectively) but constrict insignificantly at concentrations of 0.0313% or 0.0625% (4). These decreases in PD were found to occur within 15 minutes of dosing and peak pupil size reduction occurred at 30 to 60 minutes (4). On the other hand, 2% pilocarpine, although efficacious in decreasing PD by upwards of 3 mm, caused significant side effects including worsened visual field and acuity (5).

When considering the effects on PD and the potential side effects of higher doses of pilocarpine, Ocuphire found that a range of 0.3% to 0.6% pilocarpine may provide moderate efficacy with potentially limited side effects. Moreover, based on these data, Ocuphire hypothesizes that the combination of 0.4% pilocarpine, when added to phentolamine mesylate, can have an additive or synergistic effect to produce a long duration of pupil reduction in an optimal range of PD for treating presbyopia without causing undue adverse side effects due to eye redness, eye stinging, or browache.

2.3. Design Justification

Presbyopia is an age-related condition with onset most common in people over 40 years old (6). Patients experience blurred near vision, difficulty seeing in dim light, and eye strain. In young healthy eyes, lenses are able to focus light from objects at different distances by a process called accommodation. During accommodation, muscles surrounding the lens contract, causing the lens to change shape, thus increasing the focusing power of the eye. This allows for dynamic, clear vision at both near and far distances. With increasing age, the lens becomes stiffer, which limits the eye's ability to adjust its focus for reading or for other tasks that require clear vision at near distances.

Presbyopia has a significantly negative impact on quality of life (7), interfering with daily activities such as reading, use of computers or hand-held devices, and seeing the dashboard of a

car. It is estimated that 100 million Americans have presbyopia and this number is expected to grow as the population above the age of 40 increases (8).

Limitations of Existing Treatments for Presbyopia

Despite ongoing research, there are currently no noninvasive, pharmacological treatments for presbyopia that have been approved by the US Food and Drug Administration (FDA). The available treatments for presbyopia include reading glasses, bifocals, gradients, bifocal contact lenses, and multifocal intraocular lenses (IOLs). Reading glasses can be inconvenient and must be taken off and put on frequently throughout the day to see objects at far and near distances, respectively. Many patients express frustration with losing or forgetting their reading glasses. Moreover, some patients find glasses unflattering, and contact lenses for presbyopia have their own set of drawbacks regarding eye strain and limitation of use.

A small portion of patients elect surgical intervention, including laser treatment to achieve monovision and insertion of KAMRA Inlays, a plastic implant into the cornea of the non-dominant eye to increase its depth of field. The risks of such interventions are those associated with all ocular surgeries, such as a potential decrease in CS and the creation or worsening of DLD.

Nyxol Opportunity in Presbyopia

PD management for the pharmacological treatment of presbyopia is a promising strategy and an intense area of focus by several other pharmaceutical companies. Nyxol alone has shown in multiple Phase 2 trials the ability to reduce PD size by 15% to 20% and improve near VA by 1 line for at least 24 hours after a single application. Other pharmacological and device approaches have consistently demonstrated that reducing pupil size to a diameter of 1.6 to 2 mm range ("pinhole") will lead to significant improvement in presbyopia symptoms by increasing depth of focus (9-11). The creation of a pinhole-sized pupil can allow the eye to focus on near objects with less dependence on reading adds or lenses. To achieve the pinhole, Ocuphire plans to evaluate the efficacy of a kit combination of Nyxol (dosed in the evening offering a 20% reduction in PD) and LDP (dosed in the daytime with an expected additional 20% reduction in PD).

2.4. Route of Administration, Dosage Regimen, and Treatment Period

As the intended route of administration for Nyxol, Placebo, and pilocarpine is topical ophthalmic, this is the route to be used in this study.

The dose for one drop of Nyxol selected for this study, 0.75%, was based upon: 1) preclinical safety studies, 2) the results of the previous ophthalmic clinical studies described above and in the IB, and 3) clinical studies conducted with varying doses of drugs in the same class (12).

Note that 0.75% POS, which expresses the phentolamine mesylate concentration as the free base, is the new nomenclature being used in place of 1% Phentolamine Mesylate Ophthalmic Solution, which was how Nyxol had been described in prior studies, including ORION-1.

The dose for one drop of pilocarpine selected for this study is 0.4%.

All treatments will be administered OU.

2.5. Compliance

This study will be conducted in compliance with the protocol and in accordance with Good Clinical Practice (GCP), the ethical principles set forth in the Declaration of Helsinki, and with the US Code of Federal Regulations (CFR).

2.6. Study Population

The study population for this trial will be comprised of males and females between 40 to 64 years of age with presbyopia. A sample size of approximately 152 subjects will be randomized into 1 of 4 treatment arms with the expectation that approximately 140 subjects will be evaluable for efficacy.

- Nyxol + LDP
- Nyxol + No Treatment 2 (Nyxol alone)
- Placebo + LDP
- Placebo + No Treatment 2 (Placebo alone)

Subjects will be randomized 4:3:3:4 into the above groups.

After randomization, masked Treatment 1 study medications (Nyxol or Placebo) will be dispensed. Treatment 1 study medication will be taken by the subject at or near bedtime (approximately 8PM-10PM) starting the night of Visit 1 (Screening/Baseline) or at the appropriate evening in order for Treatment 1 to be taken daily for 3 to 4 consecutive days immediately prior to Visit 2.

Treatment 2 (LDP or No Treatment) is unmasked and will be administered at Visit 2 by a designated, unmasked, Site Staff member, distinct from the Site Staff member recording measurements/assessments.

Randomization will be stratified [redacted] (Appendix 1). The subjects will be recruited from approximately 20 investigational sites.

3. OBJECTIVES AND PURPOSE

The VEGA-1 study is a randomized, placebo-controlled, double-masked study of the safety and efficacy of Nyxol (0.75% POS) with 0.4% LDP in subjects with presbyopia.

The objectives of this study are:

Primary objective

- To evaluate the efficacy of Nyxol + LDP to improve DCNVA compared to Placebo alone in subjects with presbyopia

Secondary objectives

- To evaluate the efficacy of Nyxol + LDP to improve DCNVA compared to Placebo alone in subjects with presbyopia
- To evaluate the efficacy of Nyxol + LDP to prevent < 1 line of loss of BCDVA
- To evaluate the efficacy of Nyxol + LDP to improve DCNVA compared to Nyxol alone or LDP alone
- To evaluate the ability of Nyxol + LDP to produce the “pinhole” pupil size (approximately 1.6-2 mm)
- To evaluate the efficacy of Nyxol + LDP to improve BCIVA and BCDVA
- To evaluate the effect of iris color on the efficacy of Nyxol + LDP
- To evaluate the ocular and systemic safety of Nyxol + LDP and each component individually

The Sponsor intends to use this Phase 2 study to evaluate Nyxol + LDP for the chronic indication of "temporary treatment of presbyopia."

4. STUDY DESIGN

This is a placebo-controlled, double-masked, multiple-dose, Phase 2 study in approximately 152 randomized subjects with presbyopia (approximately 140 evaluable for efficacy), evaluated for safety and efficacy following administration of masked Nyxol or Placebo (Treatment 1) at or near bedtime for 3 to 4 days, with administration of LDP or No Treatment (Treatment 2) at the next visit (Visit 2) in the morning. Measurements will be made at multiple visits and timepoints, and analysis will consist of comparison across 4 treatment arms:

- Nyxol + LDP
- Nyxol + No Treatment 2 (Nyxol alone)
- Placebo + LDP
- Placebo + No Treatment 2 (Placebo alone)

Subjects will be randomized 4:3:3:4 into the above groups.

After randomization, masked Treatment 1 study medications (Nyxol or Placebo) will be dispensed. Treatment 1 study medication will be taken by the subject at or near bedtime (approximately 8PM-10PM) starting the night of Visit 1 (Screening/Baseline) or at the appropriate evening in order for Treatment 1 to be taken daily for 3 to 4 consecutive days immediately prior to Visit 2.

Treatment 2 (LDP or No Treatment) is unmasked and will be administered at Visit 2 by a designated, unmasked, Site Staff member, distinct from the Site Staff member recording measurements/assessments.

Treatment 1 (Nyxol or Placebo) will be administered to both eyes (OU) by the subject.

Treatment 2 (LDP or No Treatment) will be administered OU by the Site Staff.

The study eye is defined as the eye with worse Baseline DCNVA. In the case where both eyes have the same Baseline DCNVA, the study eye will be the right eye (OD). The non-study eye will be the fellow eye. The study eye and fellow eye will both be evaluated at all assessments.

4.1.Primary and Secondary Endpoints

Efficacy:

The primary efficacy endpoint is the percent of subjects with ≥ 15 letters of improvement in photopic binocular DCNVA on Visit 2 at 1 hour with Nyxol + LDP compared to Placebo alone. The improvement in binocular DCNVA for each subject is relative to the subject's own Baseline value (Visit 1).

Secondary efficacy endpoints (for the study eye, the fellow eye, and binocular) will include:

- [redacted]
- [redacted]

[redacted]

- [redacted]
- [redacted]

Measurements:

- DCNVA Reading/Near will be measured under photopic and mesopic conditions by a high-contrast Near Visual Acuity Chart in the [redacted] (Appendix 2)
- BCDVA will be measured under photopic conditions by a high-contrast Standard Early Treatment [redacted]
- BCIVA will be measured under photopic conditions by a high-contrast Near Visual Acuity Chart in the [redacted] at [redacted]
- PD will be measured with a [redacted] (mm)

All of the efficacy endpoints will be analyzed overall and by [redacted] at all timepoints. Subjects will also be analyzed by study eye, fellow eye, and binocular.

In photopic lighting conditions, [redacted] In mesopic conditions, [redacted]. Ambient lighting [redacted]. The [redacted]. Subjects will be allowed to acclimate to these lighting conditions (with the eyes open [redacted] to the set of PD and VA measurements). Subjects will sit in the exam chair facing directly at the illuminated chart during the acclimation period and for all assessments.

[redacted] for the scheduled remaining safety assessments (e.g., conjunctival hyperemia, AEs, subject questionnaire, etc.). The subject will be in the same room for all assessments, and every effort will be made to have the same person perform the measurements at all visits.

For VA, measurements will be made in letters and will be converted to LogMAR and lines, as appropriate.

All of the efficacy endpoints will also be analyzed by Modified Intention-to-Treat (mITT) and Per Protocol (PP) populations.

Safety:

The primary safety measures are conjunctival hyperemia, subjective ocular tolerability (Visit 2), and AEs.

Analysis for conjunctival hyperemia includes change from Baseline (Visit 1) in the conjunctival hyperemia grading scale (CCLRU images) at each visit, for study eye and fellow eye. Any increase of 2 units in conjunctival hyperemia between consecutive visits is considered an AE.

Other safety measures include IOP, biomicroscopy, ophthalmoscopy, and systemic safety as measured by HR and BP. Urine pregnancy tests for females of childbearing potential will be conducted.

Measurements:

- Conjunctival hyperemia will be assessed visually with a grading scale (0-3) using images from the CCLRU
- Subjective ocular tolerability will be measured on a 4-point scale (0-3)
- IOP will be measured with the Tono-Pen

4.2.Description and Schedule of Visits and Procedures

Study procedures are shown in detail in Table 2.

Table 2: Visit Schedule

	[redacted]
Day [b]	
Time (hr) [c]	
Informed consent	
Subject Number assigned	
Medical/Ophthalmic history	
Demographics	
Prior/Concomitant medications	
Urine pregnancy test [d]	
Heart rate/Blood pressure	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
Adverse events	
Randomization assigned [a]	
Treatment 1: Nyxol or Placebo [k]	
Treatment 2: 0.4% LDP or No Treatment [l]	
Subjective ocular tolerability [m]	

Abbreviations: BCDVA, best-corrected distance visual acuity; BCIVA, best-corrected intermediate visual acuity; CCLRU, Cornea and Contact Lens Research Unit; DCNVA, distance-corrected near visual acuity; LDP, low-dose pilocarpine; N/A, not applicable; VA, visual acuity.

[redacted].

4.3. Measures Taken to Minimize/Avoid Bias

This is a placebo-controlled, double-masked, randomized, 4-arm Phase 2 study.

4.4.Study Medications

Study Medication Identification

The POS treatment medication is phentolamine mesylate; its chemical name is 3-[N-(4,5-dihydro-1H-imidazol-2-ylmethyl)-4-methylanilino]phenol;methanesulfonic acid. It is a clean white to off-white, odorless crystalline powder with a molecular weight of 377.46 g/mol. Solutions of phentolamine mesylate are acid to litmus. It is freely soluble in water and in ethanol, and slightly soluble in chloroform. It melts at about 178ºC.

The properties (Table 3) of phentolamine mesylate are described below.

Table 3: Drug Substance and Drug Product Identifiers

Established name	Phentolamine mesylate – parent phentolamine
CAS registry number	65-28-1 – parent 50-60-2
Chemical class	An alpha-adrenergic receptor antagonist, it is a member of the following classes: imidazoles, of phenols, is a tertiary amino compound and a substituted aniline.
Chemical name	3-[N-(4,5-dihydro-1H-imidazol-2-ylmethyl)-4-methylanilino]phenol;methanesulfonic acid
Molecular formula	$C_{18}H_{23}N_3O_4S$– parent $C_{17}H_{19}N_3O$
Molecular weight (g/mol)	377.140– parent 281.352
Drug name/formulation	Nyxol / aqueous isotonic solution
Concentration active	Mesylate salt 1% – parent 0.75%
Manufacturer drug substance	[redacted]
Manufacturer drug product, Placebo	[redacted]
Storage requirements	[redacted] Stored at the site, drug will be placed in a secured location (locked) with no access for unauthorized personnel.

Nyxol (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, and sodium acetate. Placebo for Nyxol is a clear, colorless, sterile, non-preserved, isotonic, buffered aqueous solution containing mannitol and sodium acetate. [redacted] The product is preservative free.

For the proposed study, a second treatment medication (0.4% Pilocarpine Hydrochloride Ophthalmic Solution) will be used. Pilocarpine hydrochloride 1%, 2%, and 4% are approved by the FDA and available generically by multiple pharmaceutical manufacturers including Sandoz Inc, Somerset LLC, Akorn Inc, and Alcon Laboratories. For this study, a lower dose of 0.4% Pilocarpine Hydrochloride Ophthalmic Solution is planned.

Pilocarpine hydrochloride is the hydrochloride salt of (+)-pilocarpine (parent compound), a medication used to treat increased pressure inside the eye and dry mouth. Its chemical name is (3S,4R)-3-ethyl-4-[(3-methylimidazol-4-yl)methyl]oxolan-2-one hydrochloride, and it has a molecular weight of 244.72 g/mol. Pilocarpine hydrochloride is a hygroscopic, odorless, bitter-tasting white crystal or powder which is soluble in water and alcohol and virtually insoluble in most non-polar solvents. It melts at about 204.5°C. Its aqueous solubility in buffer at pH 7.4 is > 36.7 µg/mL. The parent (+)-pilocarpine is a natural alkaloid extracted from plants of the genus Pilocarpus with cholinergic agonist activity.

The properties (Table 4) of pilocarpine hydrochloride are described below.

Table 4: Drug Substance and Drug Product Identifiers

Established name	Pilocarpine hydrochloride – parent (+)-pilocarpine
CAS registry number	54-71-7 – parent 92-13-7
Chemical class	A cholinergic parasympathomimetic agent, pilocarpine predominantly binds to muscarinic receptors, thereby inducing exocrine gland secretion and stimulating smooth muscle in the bronchi, urinary tract, biliary tract, and intestinal tract. When applied topically to the eye, this agent stimulates the sphincter pupillae to contract, resulting in miosis; stimulates the ciliary muscle to contract, resulting in spasm of accommodation; and may cause a transitory rise in IOP followed by a more persistent fall due to opening of the trabecular meshwork and an increase in the outflow of aqueous humor.
Chemical name	(3S,4R)-3-ethyl-4-[(3-methylimidazol-4-yl)methyl]oxolan-2-one hydrochloride or (3S-cis)-2(3H)-Furanone, 3-ethyl-dihydro-4-[(1-methyl-1H-imidazol-5-yl)methyl] mono-hydrochloride

Molecular formula	$C_{11}H_{17}ClN_2O_2$ – parent $C_{11}H_{16}N_2O_2$
Molecular weight (g/mol)	244.72 – parent 208.261
Investigational product name/formulation	PILOCARPINE HYDROCHLORIDE 0.4% ophthalmic solution Aqueous isotonic solution, compounded by Pine Pharmaceuticals LLC and released by Iuvo BioScience LLC
Concentration active	0.4%
Drug used in compounding the investigational product	PILOCARPINE HYDROCHLORIDE 4%- pilocarpine hydrochloride solution/ drops NDC 61314-0206-15
Manufacturer drug product used for compounding	Sandoz, Inc.
Storage requirements	Stored at room temperature (15°C to 25°C, 59°F to 77°F). Stored in a secured location (locked) with no access for unauthorized personnel.

Pilocarpine hydrochloride 0.4% investigational drug is [redacted]. In addition to the active drug, the formulation also comprises the following inactive excipients: benzalkonium chloride NF, hypromellose 2910 USP, sodium citrate USP, boric acid NF, sodium chloride USP and sterile water for injection. The pH is adjusted with sodium hydroxide NF (10%) and hydrochloric acid NF (37%) to achieve a target pH of 3.5 to 5.5.

Pilocarpine hydrochloride is packaged in 15-mL LDPE dropper bottles for investigational use in the Investigator's office. Pilocarpine Hydrochloride 0.4% Ophthalmic Solution is stored at room temperature (15°C to 25°C, 59°F to 77°F).

4.4.1. Packaging and Labeling

Nyxol and its placebo are packaged in unit-of-use 1-mL low-density polyethylene (LDPE) dropper bottles with a fill volume specification of 0.6 mL for single-dose investigational use. Each bottle is wrapped with an aluminum foil overwrap impermeable to water and oxygen. Each individual pouch is 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."

LDP, one of the two options for Treatment 2, is provided in 15-mL LDPE dropper, multi-use bottles for investigational use in the Investigator's office. It 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."

4.4.2. 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.

Treatment 1 study medication (Nyxol and Placebo) should be shipped and [redacted] Treatment 1 study medication must not be frozen and must be protected from light.

Treatment 2 study medication (LDP) should be stored at room temperature (15°C to 25°C, 59°F to 77°F).

All investigational study medication must be stored in a secure facility, with access limited to the Investigator and authorized staff.

4.4.3. Study Medication Administration

Treatment 1 study medication will be distributed by the Investigator or designee at Visit 1 after randomization has been assigned. The subject will instill one drop once daily at or near bedtime (approximately 8PM-10PM) OU for 3 to 4 days.

Treatment 2 study medication will be administered by the designated, unmasked, Site Staff member, distinct from the Site Staff member recording measurements/assessments at Visit 2.

4.4.4. Study Medication Accountability

4.4.4.1. Receipt and Disposition of Study Medication

The Investigator or designee (e.g., study coordinator or pharmacist) will maintain a full accountability record for both Treatment 1 and Treatment 2 study medications and will be responsible for recording the receipt, dispensing, and return of all supplies of the study medication using the inventories supplied by Ocuphire. The Investigator or designee will account for both Treatment 1 and Treatment 2 study medications. The monitor will review dispensing and study medication accountability records during site visits and at the completion of the study and note any discrepancies.

4.4.4.2. Return of Study Medication

When the study is completed or is terminated by Ocuphire, all study material including used and unused study medication bottles will be returned to Ocuphire (or its designee) or destroyed under the direction of same. All study medication accounting procedures must be completed before the study is considered completed. A final study medication disposition will be completed by the study coordinator.

4.5. Expected Duration of Subject Participation

The total length of subject participation is approximately 5 to 9 days (accommodating for weekends) with 2 clinic visits and one telephone call follow-up as summarized below:

- Screening/Baseline Visit 1 (Day 1)
- Treatment Visit 2 (Day 4 to 6)
- Follow-up Call Visit 3 (Day 5 to 9)

The execution of the entire study (first subject screen through last randomized subject completed) is expected to take approximately 6 to 9 months.

4.6.Randomization and Procedure for Breaking the Code

At Visit 1, a randomization code for allocating subjects to Treatment 1 will be prepared by a masked biostatistician not connected with the study. Subjects will be then randomized 4:3:3:4 into 1 of 4 treatment arms:

- Nyxol + LDP
- Nyxol + No Treatment 2 (Nyxol alone)
- Placebo + LDP
- Placebo + No Treatment 2 (Placebo alone)

Randomization will be stratified 1:1 by iris color (light/dark irides).

At the initiation of study-related procedures, every subject who is 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.

The Treatment 1 study medications will be masked to Investigators, Site Staff, study subjects, the clinical research organization (CRO), and Ocuphire. 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, Ocuphire, and/or other personnel involved in the monitoring or conduct of this study.

Treatment 2 (LDP or No Treatment) is unmasked and will be administered on Visit 2 by a designated, unmasked, Site Staff member, distinct from the Site Staff member recording measurements/assessments.

Randomization for both Treatments will occur at Visit 1.

4.7.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 are 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 electronic Case Report Form (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 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.

4.8.Completed Subject

A completed subject is defined as one who completes all planned procedures through the end of Visit 2.

4.9.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.

4.9.1. Study Medication Discontinuation

The study medication may be discontinued for the following reasons:

- **Adverse Events:** AEs 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.
- **Pregnancy**: A subject may be discontinued from study medication if pregnancy occurs while on study.
- **Other:** If there is any other reason for subject discontinuation, such as pregnancy, this should be noted on the eCRF.

The reason for premature study medication discontinuation should be entered onto the appropriate eCRF.

4.9.2. Reasons for Withdrawal From Study

- Subject withdraws consent.
- Subject is lost to follow-up.
- Subject withdraws for other reason.

4.9.3. Entire Study Terminated

The entire study may be terminated by Investigators or Ocuphire. Prompt, written notice of reasonable cause to the other party (Ocuphire or Investigators, respectively) is required. Prompt notice to the Institutional Review Board (IRB) and to regulatory authorities is also required.

4.9.4. 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 non-compliant, every possible effort should be made by the Investigator to assure the 6-hour measurements are assessed prior to discontinuation, in addition to a follow-up telephone call that includes assessments for AEs, concomitant medications, and subject-evaluated conjunctival hyperemia.

4.10. Completed Study

The study is completed when all randomized subjects have completed or discontinued the study, all eCRFs have been completed, and all eCRF data entered into the database. Final database lock

will occur after the last randomized subject completes last visit, all data have been entered, and all queries resolved.

4.11.Procedure After the Completion of the Study

When the study is completed, the CRO will provide Ocuphire and the Investigator with a brief (i.e., 1-3 pages) report, containing a description of the study, the number of subjects enrolled, the number of subjects completed, the number of subjects who dropped out and why, efficacy findings, and AEs.

5. SUBJECT INCLUSION AND EXCLUSION CRITERIA

5.1 Subject Inclusion Criteria

1. Males or females ≥ 40 and ≤ 64 years of age.
2. Able to comply with all protocol-mandated procedures independently and to attend all scheduled office visits.
3. Able and willing to give signed informed consent.
4. Able to self-administer study medication throughout the study period.
5. BCDVA of 0.0 LogMAR (20/20 Snellen equivalent) or better in each eye under photopic conditions.
6. DCNVA of 0.4 LogMAR (20/50 Snellen equivalent) or worse under photopic conditions in each eye and binocularly.
7. Subjects who depend on reading glasses or bifocals in which their binocular best-corrected near VA is 0.1 LogMAR (20/25 Snellen equivalent) or better.

5.2 Subject Exclusion Criteria

Subjects excluded from the study will be individuals with the following characteristics:

Ophthalmic (in either eye):

1. Use of any topical prescription or over-the-counter (OTC) ophthalmic medications of any kind within 7 days of Screening until study completion, with the exception of lid scrubs with OTC products (e.g., OCuSOFT® lid scrub, SteriLid®, baby shampoo, etc.).
2. Use of any OTC artificial tears (preserved or unpreserved) at least once per day within 7 days of Screening until study completion.
3. Current use of any topical ophthalmic therapy for dry eye (e.g., Restasis, Xiidra, etc.).
4. Tear break-up time of < 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).
5. Clinically significant ocular disease (e.g., cataract, glaucoma, corneal edema, uveitis, retinal degeneration, loss of visual field, any macular pathology) that might interfere with the study as deemed by the Investigator.
6. Recent or current evidence of ocular infection or inflammation in either eye (such as current evidence of clinically significant blepharitis, conjunctivitis, keratitis, etc.). Subjects must be symptom free for at least 7 days.

7. Any history of herpes simplex or herpes zoster keratitis.
8. History of diabetic retinopathy or diabetic macular edema.
9. Known allergy, hypersensitivity, or contraindication to any component of the phentolamine, pilocarpine, or vehicle formulations.
10. History of cauterization of the punctum or punctal plug (silicone or collagen) insertion or removal.
11. Ocular trauma, ocular surgery (e.g., IOLs), ocular laser treatment within the 6 months prior to Screening. Any subject with multifocal IOLs are excluded.
12. History of any traumatic (surgical or nonsurgical) or non-traumatic condition affecting the pupil or iris (e.g., irregularly shaped pupil, neurogenic pupil disorder, iris atrophy, iridotomy, iridectomy, iritis, etc.).
13. Unwilling or unable to discontinue use of contact lenses at Screening until study completion.
14. Conjunctival hyperemia ≥ grade 2 on the CCLRU 4-point scale.

Systemic:

15. Known hypersensitivity or contraindication to alpha- and/or beta-adrenoceptor antagonists (e.g., chronic obstructive pulmonary disease or bronchial asthma; abnormally low BP or HR; second- or third-degree heart blockage or congestive heart failure).
16. Known hypersensitivity or contraindication to any systemic cholinergic parasympathomimetic agents.
17. Clinically significant systemic disease (e.g., uncontrolled diabetes, myasthenia gravis, cancer, hepatic, renal, endocrine, or cardiovascular disorders) that might interfere with the study as deemed by the Investigator.
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.
20. Females of childbearing potential who are pregnant, nursing, planning a pregnancy, or not using a medically acceptable form of birth control. Acceptable methods include the use of at least one of the following: intrauterine device (IUD), 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 with post-tubal ligation must have a negative urine pregnancy test result at Visit 1 (Screening/Baseline).
21. Resting HR outside the specified range of 50 to 110 beats per minute following at least a 5-minute rest period in the sitting position at the Screening Visit 1. HR may be repeated **only once** if outside the specified range, following another 5-minute rest period in the sitting position.
22. Hypertension with resting diastolic BP > 105 mmHg or systolic BP > 160 mmHg following at least a 5-minute rest period in the sitting position at the Screening Visit 1. BP may be repeated **only once** if outside the specified range, following another 5-minute rest period in the sitting position.

6. TREATMENT OF SUBJECTS

A sample size of approximately 140 subjects (40 for Nyxol + LDP and Placebo alone arms and 30 for each of the other 2 arms) who are evaluable for efficacy is needed for the study.

Eligible subjects randomized into 1 of 4 treatment arms:

- Nyxol + LDP
- Nyxol + No Treatment 2 (Nyxol alone)
- Placebo + LDP
- Placebo + No Treatment 2 (Placebo alone)

Subjects will be randomized 4:3:3:4 into the above groups. Randomization will be stratified by iris color (light/dark irides).

It is assumed that there will be approximately 7% drop-out between Visit 1 and end of Visit 2. To account for this drop-out, a total of approximately 152 subjects will be randomized into the study.

6.1. Treatment Adherence

All subjects will be instructed on the importance of following the once-daily dosing regimen for Treatment 1. Beginning the day of Visit 1 (Screening/Baseline), subjects will instill one drop of Treatment 1 study medication in each eye at or near bedtime (approximately 8PM-10PM) and repeat this procedure daily up to 3 additional times, for a total of up to 4 doses.

Effort should be made to schedule Visit 2 when the patient is onsite for Visit 1, and, if possible, Visit 2 should be scheduled within 3 or 4 days of Visit 1. If Visit 2 will be greater than 4 days after Visit 1, the subject should not start their medication until 3 or 4 days prior to Visit 2 and should take their medication for those consecutive days immediately prior to Visit 2.

Subjects are to bring unused bottles into the study site on Visit 2. Adherence to Treatment 1 will be measured by counting the dropper bottles at the start of the study and those remaining at each study visit.

Treatment 2 study medication will be administered by a designated Site Staff member, distinct from the Site Staff member recording measurements/assessments, at Visit 2.

6.2. Concomitant Medications

As noted in the exclusion criteria (Section 5.2), use of any topical prescription or OTC ophthalmic medications of any kind within 7 days of Screening, with the exception of lid scrubs with OTC products (e.g., OCuSOFT® lid scrub, SteriLid®, baby shampoo, etc.) is prohibited.

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 was no reason to believe that alteration would 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 should be documented on the appropriate eCRF. Investigators are encouraged to contact the Medical Monitor for any questions regarding allowed medications.

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 Ocuphire.

All medications that the subject has taken within 30 days prior to the Screening Visit and during the study will be recorded in the eCRF. The name of the drug, dose, route of administration, duration of treatment, and indication will be recorded for each medication. For combination and products (e.g., Contac®, Cosopt®) and non-combination products, the generic name is desired. The use of routine ophthalmic diagnostic pharmaceutical agents (e.g., fluorescein and local anesthetic) will be allowed and should be documented. Any change in dosing parameters should also be recorded in the eCRF.

7. ASSESSMENT OF EFFICACY

7.1.Specification of the Efficacy Parameters

The primary efficacy endpoint is the percent of subjects with ≥ 15 letters of improvement in photopic binocular DCNVA on Visit 2 at 1 hour with Nyxol + LDP compared to Placebo alone. The improvement in binocular DCNVA for each subject is relative to the subject's own Baseline value (Visit 1).

Secondary efficacy endpoints, which will be assessed for the study eye, the fellow eye, and binocular can be found in Section 4.1.

All of the efficacy endpoints will be analyzed overall and by light/dark irides at all timepoints.

All of the efficacy endpoints will also be analyzed by mITT and PP populations.

7.2.Assessing, Recording, and Analyzing of Efficacy Parameters

VA and PD assessments will be measured at Visit 1 (Screening/Baseline) and Visit 2 (Treatment), as described in Section 4.1.

The photopic lighting [redacted]. The mesopic lighting [redacted] When near vision is being tested, [redacted] The luminance [redacted]. Subjects will be allowed to acclimate to these lighting conditions (with the eyes open normally for a [redacted] to the set of PD, DCNVA, BCDVA, and BCIVA). Subjects will sit in the exam chair facing directly at the illuminated chart during the acclimation period and for all assessments.

Room lights may be turned on for the scheduled remaining safety assessments (e.g., conjunctival hyperemia, AEs, subject questionnaire, etc.). The subject should be in the same room for all assessments, and every effort will be made to have the same person perform the measurements at all visits.

7.2.1.Visit 1 (Screening/Baseline) – Day 1

Individuals who are potential subjects are identified by the study center to schedule the Screening Visit. The Screening Visit shall occur the same day as the Baseline Visit.

Once a subject arrives at the study center, a member of the Site Staff will interview the individual as to their qualifications for participation in the study, and if the subject wishes to continue, the informed consent form is signed, and a subject number is assigned.

The start of Screening includes an explanation of the study, a medical and ophthalmic history, and demographics. [redacted] This shall be followed by a urine pregnancy test for females of childbearing potential ([redacted]), and HR/BP ([redacted]

The subject will then undergo several VA measurements, including photopic BCDVA as well as mesopic and photopic DCNVA ([redacted] In addition, subjects will undergo measurement of photopic best-corrected intermediate visual acuity (BCIVA) and mesopic BCDVA.

The screening assessment will also include an ophthalmic examination that includes assessment of PD, biomicroscopy, dry eye examination with tear break-up time testing and corneal fluorescein staining ([redacted]), intraocular pressure (IOP) measurement (using a Tono-Pen), and direct or indirect ophthalmoscopy without dilation.

In addition, conjunctival hyperemia will be assessed visually with a 4-point grading scale using images from the CCLRU ([redacted]).

If all eligibility criteria are met, the subject will be randomized into the study.

After randomization, masked Treatment 1 study medications (Nyxol or Placebo) will be dispensed.

All treatments and assessments will be administered OU.

Please see Table 2 for details on measurements expected at Visit 1.

All treatments and assessments will be administered OU.

7.2.2. Visit 2 (Treatment 2) – Day 4

Visit 2 may occur 3 to 5 days after Visit 1. On the morning of Visit 2, subjects will undergo certain assessments immediately *prior* to receiving Treatment 2 at 0 minutes:

- Urine pregnancy test for females of childbearing potential
- HR/BP
- Concomitant medications
- [redacted]
- [redacted]

Treatment 2 (LDP or No Treatment) is unmasked and will be administered on Visit 2 by a designated, unmasked, Site Staff member, distinct from the Site Staff member recording measurements/assessments.

After instillation of Treatment 2 (LDP or No Treatment), subjects will undergo certain assessments at multiple timepoints after Time 0 (from 30 minutes to 6 hours):

- PD

- DCNVA (mesopic and photopic), BCDVA (mesopic and photopic), BCIVA (photopic) – monocular and binocular
- Conjunctival hyperemia
- Adverse events (AEs)
- Subjective ocular tolerability

Biomicroscopy, IOP, and ophthalmoscopy will be performed at the 6-hour timepoint.

There is a window of [redacted] for every timepoint to complete measurement of the primary endpoint of photopic DCNVA. Sites should start pupil diameter measurements approximately 5 minutes prior to each timepoint in order to allow sufficient time to perform the primary endpoint of photopic DCNVA at the target timepoint.

7.2.3.Visit 3 (Safety Follow-Up Call) – Day 5

For Visit 3, which will occur 1 to 3 days after Visit 2, subjects will receive a safety follow-up call to review concomitant medications and AEs.

7.2.4. 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 study participant at these visits and record any AEs in the eCRF.

As noted in Section 4.9.4, every possible effort should be made by Investigators to assure that subjects who discontinue early from the study have a telephone follow-up that includes assessments of AEs, concomitant medications, and subject-evaluated conjunctival hyperemia.

7.2.5. Visit Variation

Visit 2 may occur 3 to 5 days after Visit 1. Visit 3 occurs 1 to 3 days (any time) after Visit 2.

8. ASSESSMENT OF SAFETY

8.1.Specification of Safety Parameters

The primary safety measures are conjunctival hyperemia, subject-evaluated ocular tolerability, and AEs. Other safety measures include biomicroscopy, IOP (as measured with the Tono-Pen), and systemic safety (as measured by HR and BP). Urine pregnancy tests for females of childbearing potential will be conducted.

The assessment of safety will be evaluated by:

- Conjunctival hyperemia measured with CCLRU images using a 4-point scale (0-3) (Appendix 4).
    - None (0) = Normal. Appears white with a small number of conjunctival blood vessels easily observed
    - Mild (+1) = Prominent, pinkish-red color of both the bulbar and palpebral conjunctiva
    - Moderate (+2) = Bright, scarlet red color of the bulbar and palpebral conjunctiva
    - Severe (+3) = Beefy red with petechiae, dark red bulbar and palpebral conjunctiva with evidence of subconjunctival hemorrhage

- Subject-evaluated ocular tolerability measured on a 4-point scale.
    - 0 – No discomfort
    - 1 – Mild discomfort
    - 2 – Moderate discomfort
    - 3 – Severe discomfort
- Biomicroscopy of the anterior segment includes evaluation of lids, lashes, cornea, conjunctiva, iris, lens, and anterior chamber. Fluorescein staining will be used for tear break-up time and/or corneal fluorescein staining assessments.
- Ophthalmoscopy (direct or indirect) without PD to evaluate the vitreous and posterior pole.
- IOP is measured with the Tono-Pen.
- HR and BP (as per the site's normal equipment and procedures).
- AEs

8.2. Assessing, Recording, and Analyzing Safety Parameters

The timing for recording safety parameters may be found in Table 2.

8.3.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 TEAEs will be summarized (Section 8.3.1).

All AEs/adverse reactions occurring during the study (i.e., once the subject has signed the informed consent) **must** be documented, regardless of the assumption of causal relationship, on the respective eCRF. All TEAEs/treatment-emergent 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.

8.3.1. 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 it is considered drug related. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory findings), symptom, or disease temporarily associated with the use of the study medication, whether or not related to the study medication. Study medication includes the investigational drug under evaluation and the comparator product or vehicle placebo that is 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?" AEs also may be detected when they are volunteered by the subject during or between visits or through study assessments.

Life-threatening adverse event 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 Ocuphire, its occurrence places the subject or 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 adverse event or serious suspected adverse reaction. An AE or suspected adverse reaction is considered ''serious'' if, in the view of either the Investigator or Ocuphire, 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 (IND) 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 adverse event 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. "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."

If the relationship between the AE/SAE and the investigational product is determined by Ocuphire to be anything other than "Not related" or "Unlikely related," the event will be considered to be related to the investigational product for the purposes of expedited regulatory reporting.

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 [redacted], as well as 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 where 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.
- Unknown: Not known, not observed, not recorded, or refused.

In previous clinical studies of Nyxol, the most frequently reported AE was conjunctival hyperemia.

In the present study, any increase of 2 units in conjunctival hyperemia between consecutive timepoints is considered an AE.

Investigators are cautioned to use the appropriate verbatim term on the AE form to describe this observation:

- Redness related to instillation that is transient (i.e., is no longer present 2 hours after instillation) = “conjunctival erythema upon instillation.”
- Redness that is NOT transient (i.e., is present 2 hours after instillation) = “conjunctival hyperemia.”

Expedited reporting of serious and unexpected adverse events: All SAEs (related and unrelated) will be recorded following subject signature of the informed consent and until the Safety Follow-Up Call (Visit 3/Day 5). Any SAEs “suspected” to be related to the study medication and discovered by the Investigator at any time **after** the study must 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 eCRF. 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 VEGA1_Safety@Oculoscr.com. The Investigator must assess the SAE relationship and complete the SAE eCRF. The CRO may request additional information. Follow-up information (e.g., discharge summary) will be retained in the subject's chart and a copy will be emailed to VEGA1_Safety@Oculoscr.com. 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 Ocuphire following Ocuphire'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. Ocuphire will report all SAEs to the FDA on the appropriate schedule depending if the event is drug related or not drug related, expected, or unexpected (based on the available information in the IB).

Any death occurring during the study and follow-up 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 including the Safety Follow-Up Call, that is not reasonably associated with study medication administration, does not require completion of the SAE form.

8.3.2. 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 SAEs 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.

9. STATISTICS

9.1.Sample Size

A sample size of approximately 140 subjects (40 for Nyxol + LDP and Placebo alone arms and 30 for each of the other 2 arms) who are evaluable for efficacy is needed for the study. The primary treatment comparison will be Nyxol + LDP compared to Placebo alone. Using $\alpha = 0.05$ significance and a two-tailed test, 76 total subjects (38 subjects each in the Nyxol + LDP and Placebo alone arms) evaluable for efficacy will provide at [redacted]

Subjects will be randomized 4:3:3:4 to Nyxol + LDP, Nyxol + No Treatment 2 (Nyxol alone), Placebo + LDP, and Placebo + No Treatment 2 (Placebo alone), respectively.

It is assumed that there will be approximately 7% drop-out between Visit 1 and end of Visit 2. To account for this drop-out, a total of approximately 152 subjects will be randomized into the study.

A diagram outlining treatment arms can be found in Appendix 5.

9.2.Analysis Populations

Modified Intention-to-Treat: The mITT population will include all randomized subjects who received at least one drop of Treatment 1 and were administered Treatment 2 (LDP or No Treatment). The mITT population will be used to analyze efficacy endpoints.

Per Protocol Population: The PP population included all subjects in the mITT who received one drop of Treatment 1 the day prior to Visit 2 and were administered Treatment 2 at Visit 2, had binocular DCNVA and BCDVA in photopic conditions at Baseline (Visit 1) and at Visit 2 time 1 hour, and had no major protocol deviations. The PP population will be used for the primary endpoint analysis and to analyze efficacy endpoints.

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

Safety Population (SP): The SP will include all randomized subjects who have received at least one drop of study treatment (Treatment 1 or Treatment 2). The SP will be used to summarize safety variables.

9.3.Statistical Methods

9.3.1. General Considerations

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

All study data will be listed by treatment, subject, and timepoint (as applicable).

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.

9.3.2. Demographic and Baseline Characteristics

Demographic and baseline characteristics, such as age, race, and sex, will be summarized by treatment arm using the mITT population, PP population, the ARP, and the SP. These data will also be provided in by-subject listings.

9.3.3. Subject Disposition

Subject disposition, including randomization, and completion and withdrawal from the study will be summarized using the ARP. These data will also be provided in by-subject listings.

9.3.4. Medical History and Prior/Concomitant Medications

Medical history will be coded using the latest version of Medical Dictionary for Regulatory Activities (MedDRA, Version 23.1) and will be summarized by treatment arm using the mITT population.

Prior medications (medications with an end date prior to the date of randomization) and concomitant medications (medications with a start or end date after the date of randomization) will be coded using World Health Organization Drug Dictionary (WHODrug, Sept 2020) and will both be summarized by treatment arm using the SP.

Medical history and prior and concomitant medications will also be provided in by-subject listings.

9.3.5. Analysis of Efficacy

Efficacy will be assessed using the mITT and PP populations with subjects included in the treatment arm in which they were randomized. For the analysis of the primary efficacy endpoint, appropriate imputation techniques will be performed for missing observations if applicable; details will be provided in the study Statistical Analysis Plan. The primary comparison of interest is Nyxol + LDP and Placebo alone for the PP population. Confirmatory analyses may be performed using the ARP, with imputation performed for missing data. For the analysis of the secondary efficacy endpoints, only observed case data will be used. If warranted, confirmatory analyses using the ARP with imputation for missing data will also be performed for the secondary efficacy endpoints.

For all efficacy endpoints, Baseline values are defined as the screening assessment values on Visit 1.

All efficacy data will be summarized by treatment group, study day, and timepoint (Baseline and Visit 2), as appropriate.

The primary efficacy endpoint is the percent of subjects with ≥ 15 ETDRS letters of improvement in photopic binocular DCNVA on Visit 2 at 1 hour with Nyxol + LDP compared to Placebo alone. The improvement in binocular DCNVA for each subject is relative to the subject's own Baseline value (Visit 1). The primary efficacy endpoint will be analyzed using a logistic regression model with [redacted]
The percentage of subjects in each treatment arm meeting the criteria, the odds ratio (OR) with 95% confidence interval (CI) and p-value will be provided. The analysis will be performed using the mITT and PP populations, with subjects included in their randomized treatment regardless of the treatment they actually received. A sensitivity analysis will be completed for the primary efficacy endpoint using the same model but excluding the [redacted].

Secondary efficacy endpoints are indicated in Section 4.1.

Each of the continuous secondary efficacy endpoints will be analyzed using analysis of covariance (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. Each ANCOVA will be performed using the mITT and PP populations with subjects included in their randomized treatment regardless of the treatment they actually received. The output from each ANCOVA will include the least-squares mean (LSM) and standard error for all treatment groups, along with the placebo-corrected LSM for each treatment difference, its 95% CI, and associated p-value.

All continuous secondary endpoints derived from VA assessments, such as change in DCNVA, BCIVA, and BCDVA, will be analyzed using ETDRS letters correctly read.

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, light/dark irides, and the respective Baseline as a covariate. For each analysis, the percentage of subjects in each treatment arm meeting the criteria, the OR with 95% CI and p-value will be provided. For these endpoints, the mITT and PP populations will be used with subjects included in their randomized treatment regardless of the treatment they actually received.

In addition, each secondary efficacy endpoint will be analyzed by [redacted] using the same model indicated above but without irides as a factor, as appropriate. Other subgroups, such as age, sex, and race, may be analyzed as well.

9.3.6. Analysis of Safety

Safety will be assessed using the SP 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. A statistical analysis of conjunctival hyperemia will be conducted as described below; otherwise, no statistical analysis of safety data will be performed.

For HR and BP, Baseline is defined as the Screening value. HR and BP values and change from Baseline in the values will be summarized by treatment arm and timepoint (Visit 1 and Visit 2).

Observed values and change from Baseline (Visit 1) in conjunctival hyperemia at each timepoint (Visit 1 at 0 and 1 hour, and Visit 2 at 0 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 6 hours), will be summarized for the study eye and fellow eye. Treatments will be compared using the same ANCOVA model proposed for the continuous secondary efficacy endpoints.

For IOP, Baseline is defined as the Screening value. Observed values and change from Baseline in IOP at each timepoint (Visit 1 and Visit 2) will be summarized for the study eye and the fellow eye.

Ocular tolerability values will be summarized by treatment arm at each timepoint (Visit 1 and Visit 2).

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. TEAEs and SAEs will be summarized by treatment group, by system organ class (SOC), severity, and relationship to study medication. Deaths, withdrawal from study medication due to AEs, and withdrawal from the study due to AEs will each by summarized by treatment group. 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.

9.4. Procedure for Accounting for Missing, Unused, or Spurious Data

For the summarization and analysis of efficacy data, the focus will be on observed case data only. As appropriate, confirmatory efficacy analyses will be performed using imputation for missing data; details of the imputation, if performed, will be included in the study Statistical Analysis Plan. For the summarization of safety data, observed case data only will be used.

9.5.Procedure for Reporting Deviations From the Statistical Plan

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

10. 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.

11. 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 COVID-19 pandemic restrict 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 Ocuphire, the Study Monitor, and the Medical Monitor to inspect all eCRFs, subject records (source documents), signed consent forms, records of study medication receipt, storage, preparation, and disposition, and regulatory files related to this study.

12. ETHICAL CONSIDERATIONS AND GCP COMPLIANCE

12.1.GCP 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) GCP guidelines, obtaining prospective informed consent, monitoring of the conduct of the study and the completeness of the eCRFs by Ocuphire or its designee(s), and appropriate record retention by the Investigator.

12.2.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 Ocuphire, *prior* to the start of subject enrollment into the study.

12.3.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 (e.g., 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, Ocuphire or 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.

12.4. 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 informed consent document 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 who withdraw written informed consent may not be included or continued in this study, but this will not impact on 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 will be required to sign and date the informed consent form.

A copy of the signed and dated consent document will be given to each subject. The original signed and dated informed consent document 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.

13. 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.

13.1.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.

13.2.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. CRAs 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.

13.3.Archiving of Data

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

13.4. Records Retention

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

13.5.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 Ocuphire 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 (e.g., change in monitor, change of telephone number).

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

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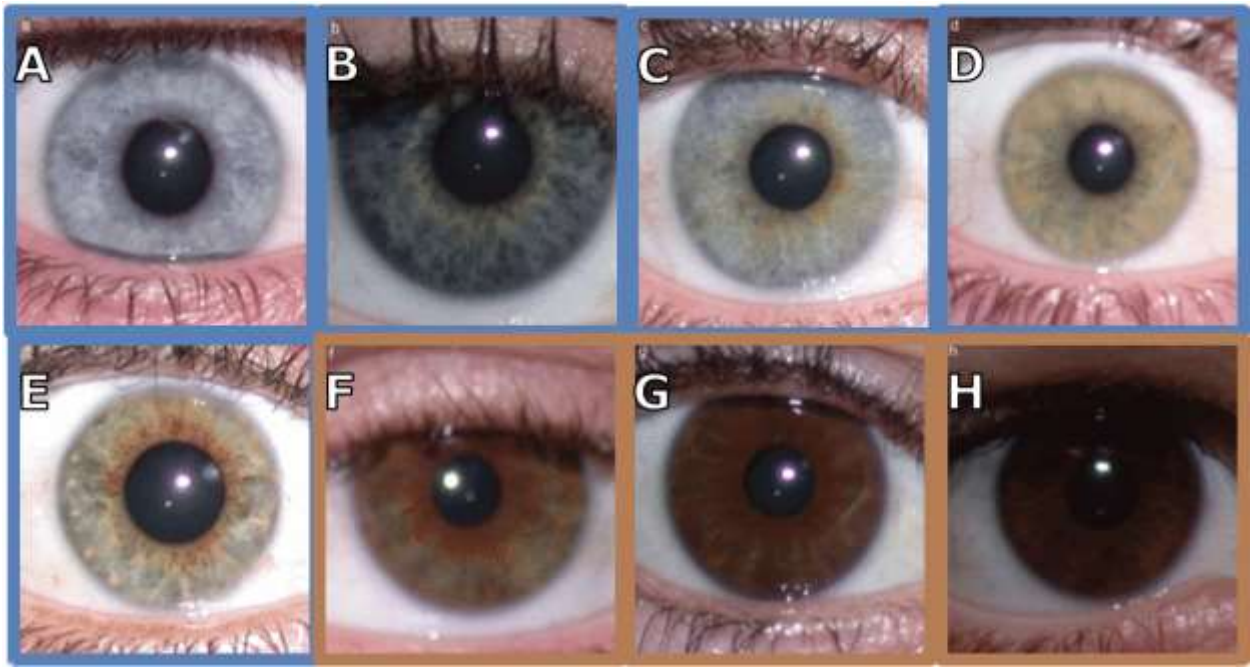
APPENDIX 1: IRIS COLOR CHART

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: VISUAL ACUITY CHARTS

DCNVA will be measured using the Near Visual Acuity Chart (logarithmic Visual Acuity Chart 2000, chart #1 and #2) in the Precision Vision Small 914 Illuminator Cabinet (light box) at 16 inches (~40 cm) (letters recorded, later converted to LogMAR and number of lines).

Near High-Contrast Chart

BCDVA will be measured with a Standard ETDRS illuminated 100% high-contrast chart (on wall or stand) at 4 m.

Distance High-Contrast Chart

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. ***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 ***Alpha-2-agonists*** Brimonidine Clonidine Guanfacine Guanabenz Guanoxabenz Guanethidine Xylazine Tizanidine Methyldopa	***Non-selective alpha-antagonists*** Phenoxybenzamine Tolazoline Labetalol Carvedilol ***Alpha-1-antagonists*** Alfuzosin Prazosin Doxazosin Tamsulosin Terazosin ***Alpha-2-antagonists*** Atipamezole Idazoxan Yohimbine	***Acetylcholine receptor agonists*** Pilocarpine (M_3 receptors) ***Acetylcholine receptor antagonists*** Scopolamine Dicycloverine Tolterodine Oxybutynin Ipratropium Mamba Toxin (MT_7) Pirenzepine Telenzepine ***Antivertigo*** Meclizine Scopolamine	***Gastrointestinal*** Atropine Belladonna ***Parkinsonism*** Amantadine Benztropine Biperiden Trihexyphenidyl

APPENDIX 4: CONJUNCTIVAL HYPEREMIA GRADING SCALE USING IMAGES FROM CCLRU

			
None (0)	**Mild (+1)**	**Moderate (+2)**	**Severe (+3)**
Normal. Appears white with a small number of conjunctival blood vessel easily observed	Prominent, pinkish-red color of both the bulbar and palpebral conjunctiva	Bright, scarlet red color of the bulbar and palpebral conjunctiva	Beefy red with petechiae, dark red bulbar and palpebral conjunctiva with evidence of subconjunctival hemorrhage

APPENDIX 5: TREATMENT GROUPS