CONFIDENTIAL

Clinical Study Phase 3 Protocol
OPI-NYXP-301
VEGA-2

Randomized, Double-Masked, Placebo-Controlled, Multicenter, Phase 3 Study of the Safety and Efficacy of Nyxol (Phentolamine Ophthalmic Solution 0.75%) as a Single Agent and With Adjunctive Low-Dose Pilocarpine Hydrochloride Ophthalmic Solution 0.4% in Subjects With Presbyopia

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Original:	**July 08, 2022**
Amendment 1:	**October 31, 2022**
Amendment 2:	**November 15, 2022**
Amendment 3:	**September 29, 2023**

SPONSOR SIGNATURE & CONTACTS

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

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[redacted]

[redacted]

Date

1. SYNOPSIS

Study Number	OPI-NYXP-301
Clinical Phase	Phase 3
Type of Study	Randomized, double-masked, placebo-controlled, multicenter study of the safety and efficacy of Nyxol® (Phentolamine Ophthalmic Solution [POS] 0.75%) as a single agent and with adjunctive Pilocarpine Hydrochloride Ophthalmic Solution 0.4% (Low-Dose Pilocarpine, LDP) in subjects with presbyopia
Name of Investigational Products	Nyxol Eye Drops – POS 0.75% LDP 0.4%
Duration of Study	22 to 36 days
Study Objectives	[redacted]

Design	[redacted]

	[redacted]
Study Drug Dispensing and Treatment Adherence	[redacted]

	[redacted]
Subject Population	Approximately 320 randomized subjects with presbyopia
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. [redacted] 8. [redacted]
Exclusion Criteria	**Ophthalmic (in either eye):** 1. Use of any topical prescription (including Vuity™) 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 (eg, OCuSOFT® lid scrub, SteriLid®, baby shampoo, etc.) and artificial tears as specified in Exclusion #2 below. 2. Use of any OTC artificial tears (preserved or unpreserved) during Visit days or 15 min before or after instillation of Treatment 1 or Treatment 2. 3. Current use of any dry eye product such as topical ophthalmic therapy for dry eye (eg, generic cyclosporine, Restasis, Xiidra, Cequa, and Eysuvis) or intranasal dry eye product (eg, Tyrvaya) or other devices. 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 (eg, cataract, glaucoma, corneal edema, uveitis, retinal degeneration, loss of visual field, or 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. Known allergy, hypersensitivity, or contraindication to any component of the phentolamine, pilocarpine, or vehicle formulations. 9. Prior participation in a study involving the use of Nyxol for the treatment of presbyopia. 10. History of cauterization of the punctum or punctal plug (silicone or collagen) insertion or removal. 11. Ocular trauma within 6 months prior to Screening. 12. Ocular surgery or any ocular laser treatment within 6 months prior to Screening. 13. Subjects with surgical monovision, multifocal or extended depth of focus intraocular lenses (IOLs) are excluded (monofocal IOLs are accepted if in place > 6 months prior to Screening). 14. History of any traumatic (surgical or nonsurgical) or nontraumatic condition affecting the pupil or iris (eg, irregularly shaped pupil, neurogenic pupil disorder, iris atrophy, iridotomy, iridectomy, iritis, etc.). 15. Contact lens wear on the day of any study visit and contact lenses must be removed for home dosing and for at least 10 minutes following dosing. **Systemic:** 16. Known hypersensitivity or contraindication to alpha- and/or beta-adrenoceptor antagonists (eg, chronic obstructive pulmonary disease or bronchial asthma; abnormally low blood pressure [BP] or heart rate [HR]; second- or third-degree heart blockage or congestive heart failure). 17. Known hypersensitivity or contraindication to any systemic cholinergic parasympathomimetic agent. 18. Clinically significant systemic disease (eg, uncontrolled diabetes, myasthenia gravis, cancer, hepatic, renal, endocrine, or cardiovascular disorders) that might interfere with the study as deemed by the judgment of the Investigator. 19. 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; however, Flomax (tamsulosin) is specifically excluded. 20. Participation in any investigational study within 30 days prior to Screening. 21. 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). 22. Resting HR outside the range of 50 to 110 beats per min following at least a 5-min rest period in the sitting position at Visit 1 (Screening/Baseline). Heart rate may be repeated **only once** if outside the specified range, following another 5-min rest period in the sitting position. 23. Hypertension with resting diastolic BP > 105 mmHg or systolic BP > 160 mmHg following at least a 5-min rest period in the sitting position at Visit 1 (Screening/Baseline). Blood pressure may be repeated **only once** if outside the specified range, following another 5-min rest period in the sitting position.
Study Assessments	BCDVA [redacted] Distance-corrected intermediate visual acuity (DCIVA) [redacted] DCNVA [redacted] PD [redacted] Biomicroscopy Intraocular pressure (IOP) Direct or indirect ophthalmoscopy without dilation Urine pregnancy test for females of childbearing potential HR and BP Ocular tolerability Subject questionnaire Corneal fluorescein staining (for eligibility only)

Number of Investigational Sites	Approximately 30 sites
Estimated Total Sample Size	Approximately 320 randomized subjects
Sample Size Justification	Using α = 0.05 significance and a two-tailed test, 300 total subjects (150 subjects each in the Nyxol and placebo arms) who are evaluable for efficacy [redacted] in percent of subjects with ≥ 15 letters of improvement in photopic binocular DCNVA and with < 5 letters of loss in photopic binocular BCDVA from Baseline at 12 hours post-dose at Visit 2 (Stage 1 Day 8). This calculation is based on [redacted]. An additional 20 subjects will be enrolled to account for dropouts.
Primary Efficacy Endpoint	The primary efficacy endpoint is the percent of subjects with ≥ 15 letters of improvement in photopic binocular DCNVA and with < 5 letters of loss in photopic binocular BCDVA at 30 min post-LDP/vehicle comparing Nyxol + LDP to placebo + LDP vehicle at Visit 5 (Stage 2 Day 8). This comparison will be analyzed first using a hierarchical testing method.
Key Secondary Efficacy Endpoints	[redacted]
Other Secondary Efficacy Endpoints	[redacted]

[redacted]	[redacted]

	[redacted] All efficacy endpoints will be analyzed overall and by light/dark irides. Separate analyses will be performed for binocular, study eye, and fellow eye, where applicable. All efficacy endpoints will also be analyzed by the Modified Intention-to-Treat (mITT) Population. Selected analyses will be performed on the Per Protocol (PP) Population as specified in the Statistical Analysis Plan (SAP). [redacted]
Safety Endpoints	Safety measures are adverse events, ocular tolerability, IOP, BCDVA, biomicroscopy, ophthalmoscopy, HR and BP, and subject questionnaire. Urine pregnancy tests for females of childbearing potential will be conducted.
Study Medications, Dose, and Mode of Administration	Treatment 1: • Nyxol® Eye Drops (Phentolamine Ophthalmic Solution 0.75%), or • Placebo (ie, Nyxol vehicle) Treatment 1 is dosed during both Stages 1 and 2 of the study and is taken daily in the evenings near bedtime, except during the Washout Period. Treatment 2: • LDP (Pilocarpine Hydrochloride Ophthalmic Solution 0.4%), or • LDP vehicle Treatment 2 is dosed during Stage 2 of the study and is taken daily in the mornings.

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2. ABBREVIATIONS AND TERMS

Abbreviation	*Full term*
AE	adverse event
ANCOVA	analysis of covariance
ARP	All Randomized Population
BCDVA	best-corrected distance visual acuity
BP	blood pressure
cGMP	current Good Manufacturing Practice
CI	confidence interval
CRA	clinical research associate
CRO	contract research organization
DCNVA	distance-corrected near visual acuity
eCRF	electronic Case Report Form
ETDRS	Early Treatment Diabetic Retinopathy Study
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HR	heart rate
IB	Investigators' Brochure
ICH	International Council for Harmonisation
IOL	intraocular lens
IOP	intraocular pressure
IRB	Institutional Review Board
ITT	Intention-to-Treat
IUD	intrauterine device
LDP	Low-Dose Pilocarpine Hydrochloride Ophthalmic Solution 0.4%; Low-Dose Pilocarpine
LDPE	low-density polyethylene
LSM	least-squares mean
MedDRA	Medical Dictionary for Regulatory Activities

mITT	Modified Intention-to-Treat
Nyxol	Phentolamine Ophthalmic Solution 0.75%
Nyxol + LDP	Nyxol dosed in the evenings with LDP dosed during the mornings
OD	oculus dexter (right eye)
OR	odds ratio
OS	oculus sinister (left eye)
OTC	over-the-counter
OU	oculus uterque (both eyes)
PCH	pilocarpine hydrochloride
PD	pupil diameter
POS	Phentolamine Ophthalmic Solution
PP	Per Protocol
PPS1	PP Protocol Population Stage 1
PPS2	PP Population Stage 2
SAE	serious adverse event
SAP	Statistical Analysis Plan
SOC	system organ class
SP	Safety Population
TEAE	treatment-emergent adverse event
US	United States
USP	United States Pharmacopeia
VA	visual acuity

3. INTRODUCTION

In this study, the efficacy of Nyxol (Phentolamine Ophthalmic Solution [POS] 0.75%) as a single agent and the combination of Nyxol and Pilocarpine Hydrochloride Ophthalmic Solution 0.4% (Low-Dose Pilocarpine, LDP) in improving distance-corrected near visual acuity (DCNVA) will be evaluated in presbyopic subjects.

3.1. Findings From Nonclinical and Clinical Studies

Nyxol

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

Nyxol has been studied in a total of 12 clinical trials (three Phase 1, five Phase 2, and four Phase 3) enrolling over 1100 subjects (including subjects 3 years of age and up, with over 650 subjects treated with Nyxol) and has demonstrated promising clinical data for use in multiple ophthalmic indications.

In prior clinical trials, Nyxol has demonstrated a consistent ability to decrease pupil diameter (PD) by approximately 20% - 30% (~1-1.5 mm) in both mesopic and photopic conditions (1-3). Nyxol has been found to be efficacious for more than 24 hours, with an onset of action of approximately 30 to 60 min. Nyxol has been observed to be well tolerated at single doses and multiple doses up to 14 days. Safety of the subjects in these trials was evaluated by adverse event (AE) monitoring, physical examinations, and vital sign assessments. Across all trials, no serious adverse events (SAEs) have been reported. No deaths occurred in any of the trials. No clinically meaningful changes were observed in physical examinations or vital signs, including blood pressure (BP) and heart rate (HR). Adverse events reported were mild to moderate in intensity, with the most common being transient conjunctival hyperemia (eye redness) and ocular irritation; however, Nyxol dosing at or near bedtime was observed to mitigate or minimize these side effects during the daytime.

The VEGA-1 study evaluated the efficacy and safety of Nyxol in combination with LDP and as a single agent in presbyopic subjects. The study met its primary and many secondary endpoints.

Nyxol + LDP showed a favorable safety profile with no serious AEs. Most AEs were mild, with no headaches, no browaches, and no blurry vision AEs reported. Mild, transient conjunctival hyperemia AEs were reported in < 5% of subjects.

Pilocarpine Hydrochloride

A detailed report of pilocarpine, its product label, properties, and potential risk are provided in the IB (2022).

Pilocarpine hydrochloride (PCH) has been studied in a prior clinical trial by Ocuphire. In addition, there is a recently approved PCH drug at 1.25% and extensive literature on pilocarpine's ability to reduce PD across various concentrations. These pupil-constricting effects, however, 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 PCH that would have minimal side effects, Ocuphire conducted an expansive review of the medical literature concerning experiments of various doses of PCH and their effects on PD and side effect profile. Leavitt et al. found that

normal pupils constrict with dilute concentrations of 0.25% and 0.125% pilocarpine (1.7 and 0.6 mm, respectively) but constrict insignificantly at concentrations of 0.0313% and 0.0625% (4). These decreases in PD were found to occur within 15 min of dosing, and peak pupil size reduction occurred at 30 to 60 min (4). On the other hand, 2% pilocarpine, although efficacious in decreasing PD by up to 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% PCH may provide moderate efficacy with potentially limited side effects. Moreover, based on these data, Ocuphire has shown in its Phase 2 VEGA-1 study that the combination of Nyxol and LDP can have an additive to synergistic effect to produce a long duration of pupil reduction in an optimal range of PDs for treating presbyopia, without causing adverse side effects such as headache, browache, or accommodative spasm.

3.2. 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, the ciliary muscle surrounding the lens contracts, 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

Vuity™ (PCH 1.25%) has recently been approved for the treatment of presbyopia. In two Phase 3 studies, Vuity met its primary outcome measure, with 31% and 26% of Vuity-treated subjects gaining 3 or more lines of mesopic DCNVA 3 hours post-dose compared to 8% and 11% of placebo-treated subjects. However, limitations to Vuity include limited duration of efficacy, with a need for twice daily dosing to achieve efficacy throughout the day; headache, browache, and burning/stinging associated with drug administration; accommodative spasm and risk of angle-closure glaucoma due to activation of the ciliary muscle; and risk of retinal detachment in subjects with high myopia (9).

Other 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. The risks of such interventions include a potential decrease in contrast sensitivity and stereopsis and the creation or worsening of dim light vision disturbances.

Nyxol Opportunity in Presbyopia

Pharmacological treatment of presbyopia is a promising strategy and an intense area of focus by several pharmaceutical companies. Other pharmacological and device approaches have consistently demonstrated that reducing pupil size to a diameter of approximately 2 to 3 mm will lead to significant improvement in presbyopia symptoms by increasing depth of focus (10-12). In multiple trials, it has been shown that Nyxol reduces PD by approximately 20% to 30% and improves near visual acuity (VA) for at least 24 hours after a single application. OPI-NYXP-201 (VEGA-1) was a 7-day (2 visits), double-masked, randomized, placebo-controlled, multicenter Phase 2 trial in 150 presbyopic subjects. Results of this study suggest that treatment with Nyxol alone provides a ≥ 3-line benefit in DCNVA in a similar percent of subjects to that observed with Vuity, with better durability and a better safety profile. Nyxol with adjunctive LDP therapy resulted in a higher percentage of presbyopic subjects achieving a gain of 3 or more lines of DCNVA, with increased durability and a better safety profile compared to those from the Vuity pivotal trials. A summary of the results of the VEGA-1 study are presented here.

Efficacy Results From the VEGA-1 Study:

Nyxol as a Single Agent

Following 3 to 4 days of treatment with Nyxol or placebo, 30.1% of Nyxol-treated subjects had ≥ 15 letters of improvement in photopic binocular DCNVA compared with 13.5% of placebo-treated subjects 12 hours after the last dose (p=0.0265). This benefit remained stable over the next 6 hours, with a mean of 36.7% of Nyxol-treated subjects improving by 15 or more letters DCNVA 18 hours post-dose [redacted] Significantly more subjects treated with Nyxol achieved binocular DCNVA of 20/40 or better compared with placebo (56% vs 36%, respectively; p=0.0080).

Photopic binocular mean DCNVA showed significantly greater improvement in mean number of letters read among Nyxol-treated subjects (10.1-letter improvement) compared with placebo-treated subjects (5.6-letter improvement) at 12 hours post-dose (p=0.0006). Consistent with these results, mean photopic PD in the best eye was significantly reduced among Nyxol-treated subjects compared with placebo-treated subjects at 12 hours post-dose (p<0.0001).

Nyxol + LDP as Adjunctive Therapy

A statistically significantly greater number of subjects in the Per Protocol (PP) Population treated with Nyxol + LDP had ≥ 15 letters of improvement in photopic binocular DCNVA at 1 hour post-LDP treatment (primary endpoint) compared with placebo alone (60.45% vs 27.9%, respectively; p=0.0037). In addition, the Nyxol + LDP arm had significantly more subjects with ≥ 15 letters of improvement in photopic binocular DCNVA compared with placebo alone at all timepoints from 30 min through 4 hours post-LDP treatment (p≤0.0166 for each). This benefit was retained for all timepoints between 30 min and 4 hours when requiring that subjects with ≥ 15 letters of improvement in photopic binocular DCNVA also had to have < 5 letters of loss in photopic binocular best-corrected distance visual acuity (BCDVA), the accepted registration endpoint. The benefit of LDP adjunctive therapy to Nyxol was consistent regardless of iris color.

The effect of Nyxol + LDP on DCNVA was rapid and clinically significant. By 30 min post-LDP treatment, 79% of subjects had photopic binocular DCNVA of 20/40 or better. By 1 hour post-LDP

treatment, 84% of subjects had binocular DCNVA of 20/40 or better. Significantly more subjects treated with LDP adjunctive to Nyxol had photopic binocular DCNVA 20/40 or better compared with placebo at 30 min through 2 hours ($p \leq 0.0119$ for each).

Nyxol + LDP treatment resulted in more subjects achieving a ≥ 15-letter improvement in DCNVA compared with each of its components used individually at all timepoints from 30 min to 2 hours post-LDP treatment. Although the study was not statistically powered for this outcome, the percent of responders at 30 min with combination therapy (61%) was significantly greater than the response rate with LDP alone (26%; $p=0.0076$) or Nyxol alone (33%; $p=0.0265$).

Safety Results From the VEGA-1 Study:

Nyxol, when administered with LDP as adjunctive therapy or as a single agent, was well tolerated in this study. Fifty treatment-emergent adverse events (TEAEs) were reported in 15 subjects (34.1%) treated with Nyxol + LDP, 4 TEAEs were reported in 2 subjects (4.4%) treated with placebo alone, 18 TEAEs were reported in 6 subjects (20.0%) treated with Nyxol alone, and 13 TEAEs were reported in 6 subjects (19.4%) treated with placebo + LDP. All subjects experienced mild TEAEs except for 3 subjects: 1 in the placebo alone arm and 2 in the Nyxol + LDP arm. All subjects had TEAEs that were considered possibly, probably, or definitely related to treatment, except for 2 subjects: 1 in the placebo alone arm had an unrelated TEAE and 1 in the Nyxol + LDP arm had a TEAE of unknown relatedness. One subject in the Nyxol + LDP arm had 2 TEAEs (mild conjunctival hyperemia and mild eye irritation) leading both to study medication discontinuation and withdrawal from the study. No subjects had any serious TEAEs, and no subjects died during the study.

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

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

The dose for 1 drop of Nyxol (POS 0.75%) selected for this study 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 (13).

Note that POS 0.75%, which expresses the phentolamine mesylate concentration as the free base, is the new nomenclature being used in place of Phentolamine Mesylate Ophthalmic Solution 1%, which was how Nyxol had been described in studies prior to 2020.

The dose for 1 drop of Pilocarpine Hydrochloride Ophthalmic Solution selected for this study is 0.4% (LDP).

All treatments will be administered in both eyes (OU).

3.4. 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 United States (US) Code of Federal Regulations.

3.5. Study Population

The study population for this trial will be comprised of males and females between 40 and 64 years of age with presbyopia. A sample size of approximately 320 subjects will be randomized into 1 of

4 treatment arms, with the expectation that approximately 300 subjects will be evaluable for efficacy.

- Nyxol + LDP
- Nyxol + LDP vehicle
- Placebo (ie, Nyxol vehicle) + LDP
- Placebo + LDP vehicle

Subjects will be randomized 1:1:1:1 into the above groups.

At Visit 1 (Screening/Baseline), eligible subjects will be randomized, and the first dose of Treatment 1 will be administered (note: if Visit 1 is split into 2 separate days, then dosing occurs on the second day). Efficacy and safety measurements are made before dosing with Treatment 1 (Time 0 min) and at multiple post-dose timepoints from 30 min to 8 hours. If Visit 1 is split over 2 separate days, all Screening assessments up to and including randomization must occur on the first day, and then the subject should return to complete their post-randomization assessments in the following order on the second day (ie, confirm concomitant medications, subject questionnaire, in-office Treatment 1 dosing, ocular tolerability, followed by all other assessments at time points 0.5, 1, 3, 5, and 8 hours post-treatment). Prior to discharge following completion of Visit 1 (Stage 1 Day 1), Treatment 1 study medication for Stages 1 and 2 will be dispensed for evening dosing by the subject before bedtime, to begin the night of Stage 1 Day 2. On the evening prior to Visit 2, the subject will dose Treatment 1 study medication as close to 12 hours prior to Time 0 min for that visit.

At Visit 2 (Stage 1 Day 8), efficacy and safety measurements will be made at 12 hours after the dose of Treatment 1 study medication (12-hour endpoint). After completion of these assessments, **the subject will take no study drug for 7 to 14 days (during the Washout Period)**, after which Stage 1 is complete. Measurements will be made on Visit 3 (Stage 1 Day 10 ± 1 day) during the Washout Period to assess the absence of residual treatment effects.

Subjects will restart dosing daily with Treatment 1 in the evening immediately prior to Visit 4 (Stage 2 Day 1) as close to 12 hours prior to the scheduled time for Visit 4 for the 12-hour assessments. At this visit, after completion of the 12-hour assessments for Treatment 1, subjects will be administered their morning dose of Treatment 2 (at Time 0 min) at the study site, and efficacy and safety measurements will be made at multiple post-dose timepoints over the next 8 hours. During Stage 2, subjects will administer Treatment 1 in the evenings near bedtime and Treatment 2 in the mornings from the evening prior to Visit 4 (Stage 2 Day 1) for the next 7 days or until the evening before their scheduled Visit 5 (Stage 2 Day 8). On the evening prior to Visit 5, Treatment 1 should be administered as close to 12 hours prior to the scheduled time for Visit 5. At Visit 5, measurements will be made before dosing with Treatment 2 at Time 0 min and at multiple post-dose timepoints over 8 hours after dosing with Treatment 2.

[redacted] Appendix 1 The number of subjects in each irides type should be as close as possible to 1:1, with a minimum of 40% within each stratum. The subjects will be recruited from approximately 30 investigational sites in the US.

4. OBJECTIVES AND PURPOSE

The VEGA-2 study is a randomized, placebo-controlled, double-masked study of the safety and efficacy of Nyxol (POS 0.75%) in combination with LDP 0.4% in subjects with presbyopia.

The objectives of this study are:

Primary objective

- To evaluate the efficacy of LDP as adjunctive therapy to Nyxol (Nyxol + LDP) to improve DCNVA without loss of BCDVA compared to placebo + LDP vehicle in subjects with presbyopia

Additional objectives

[redacted]

he Sponsor intends to use this Phase 3 pivotal study to evaluate Nyxol as a single agent and Nyxol + LDP for [redacted]

5. STUDY DESIGN

This is a randomized, double-masked, placebo-controlled, multicenter, Phase 3 study in approximately 320 subjects with presbyopia. The study will be performed in 2 stages, as shown in Appendix 4.

Subjects will be randomized 1:1:1:1 to treatments for both stages at Visit 1 (Screening/Baseline). Treatment 1 assignment in Stage 1 will be maintained for Stage 2. Stage 1 will consist of 2 treatment groups (Nyxol or placebo [ie, Nyxol vehicle]), with approximately 160 subjects in each group. Stage 2 consists of 4 treatment groups (Nyxol + LDP, Nyxol + LDP vehicle, placebo + LDP, and placebo + LDP vehicle), with approximately 80 subjects per treatment group.

[redacted] Appendix 1 [redacted]. The subjects will be recruited from approximately 30 investigational sites in the US.

Stage 1: Dosing of Treatment 1

In Stage 1, the safety and efficacy of Nyxol as a single agent will be evaluated over the following 1 day and 7 days of daily dosing in the evenings near bedtime, except the evening before Visit 2, when Treatment 1 is dosed as close to 12 hours prior to Time 0 min for that visit. Following Visit 2 (Stage 1 Day 8), subjects will discontinue dosing Treatment 1 for 7 to 14 days (Washout Period). During the Washout Period, subjects will return for Visit 3 (Stage 1 Day 10 ± 1 day) to assess the resolution of drug treatment effects.

During Stage 1, subjects will dose with Treatment 1 (Nyxol or placebo [ie, Nyxol vehicle]) in the evenings near bedtime (except for Visit 1 [Stage 1 Day 1], when the subject is dosed during the day at the study site).

Measurements will be made at multiple visits and timepoints, and analysis will consist of comparison across the 2 arms:

- Nyxol
- Placebo

Stage 2: Dosing of Treatments 1 and 2

Visit 4 (Stage 2 Day 1) is the start of Stage 2. In Stage 2, the efficacy and safety of Nyxol + LDP will be evaluated following 1 day and 7 days of dosing, in which Treatment 1 (Nyxol or placebo) is dosed daily in the evenings near bedtime, except the evening before Visits 4 and 5, when Treatment 1 is dosed as close to 12 hours prior to the scheduled time for those visits. Treatment 2 (LDP or LDP vehicle) is dosed daily in the mornings. Treatment 2 will be dosed at the study site at the beginning of Visits 4 and 5 (Stage 2 Days 1 and 8, respectively).

Measurements will be made at multiple visits and timepoints, and analysis will consist of comparison across the 4 arms:

- Nyxol + LDP
- Nyxol + LDP vehicle
- Placebo + LDP
- Placebo + LDP vehicle

Other design considerations:

Study medications (Treatments 1 and 2) will each be dosed as a single drop in each eye, with Treatment 1 dosed in the evenings near bedtime and Treatment 2 dosed in the mornings. Baseline for the study is defined as pre-treatment up to and including randomization (prior to Time 0 min) at Visit 1 (Screening/Baseline).

During Visit 2 (Stage 1 Day 8), Time 0 min is defined as the timing of the first efficacy assessments at the visit, approximately 12 hours after dosing with Nyxol the previous night (12-hour post-dose timepoint). During Visits 4 and 5 (Stage 2 Days 1 and 8, respectively), Time 0 min is defined as the time of Treatment 2 administration.

The study eye is defined [redacted] The study eye and fellow eye will both be

evaluated at all assessments. The baseline values are determined during Screening assessments at Visit 1.

5.1. Primary And Secondary Endpoints

Efficacy:

The primary efficacy endpoint is the percent of subjects with ≥ 15 letters of improvement in photopic binocular DCNVA and with < 5 letters of loss in photopic binocular BCDVA at 30 min post-LDP/vehicle comparing Nyxol + LDP to placebo + LDP vehicle at Visit 5 (Stage 2 Day 8).

This comparison will be analyzed first using a hierarchical testing method.

The [redacted]

[redacted]

Measurements:

- Best-corrected distance VA will be measured in photopic conditions by a high-contrast Standard Early Treatment Diabetic Retinopathy Study (ETDRS) illuminated chart (on wall or stand) at 4 m
- Distance-corrected intermediate VA will be measured [redacted] by a high-contrast Near Visual Acuity Chart in the Precision Vision Small 914 Illuminator Cabinet (light box) at 26 inches (66 cm)
- Distance-corrected near VA will be measured in [redacted] by a high-contrast Near Visual Acuity Chart in the Precision Vision Small 914 Illuminator Cabinet (light box) at 16 inches (~40 cm) (Appendix 2)

[redacted]

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

In [redacted]

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 efficacy endpoints will also be analyzed by the Modified Intention-to-Treat (mITT) Population and selected efficacy endpoints will be analyzed using the PP Population as specified in the Statistical Analysis Plan (SAP).

Safety:

Safety measures are AEs, ocular tolerability, intraocular pressure (IOP), BCDVA, biomicroscopy, ophthalmoscopy, HR and BP, and subject questionnaire. Urine pregnancy tests for females of childbearing potential will also be conducted.
Measurements:

- Biomicroscopy, ophthalmoscopy, and HR and BP measured in accordance with the site's standard practice
- Ocular tolerability will be measured on a 4-point scale (0-3)
- Intraocular pressure will be measured with a Tono-Pen or Goldmann Applanation tonometry
- Subject questionnaire will be completed by the subject (Appendix 5 and Appendix 6)

5.2. Description and Schedule of Visits and Procedures

Study procedures are shown in detail in the Schedule of Events tables (Table 1 and Table 2).

Table 1: Schedule of Events for Stage 1

	Screening/Baseline Visit 1[a]						Visit 2[b]							Visit 3[b]
Day	**Stage 1 Day 1**						**Stage 1 Day 8**						**Washout Period 7 – 14 days**	**Stage 1 Day 10** (± 1 day)
Time (hr)[c]	**0**	**0.5**	**1**	**3**	**5**	**8**	**0**	**0.5**	**1**	**3**	**5**	**8**		
Informed consent	X													
Subject ID # assigned	X													
Medical/Ophthalmic history	X													
Demographics	X													
Prior/Concomitant medications	X*						X							X
Urine pregnancy test[d]	X						X							
HR/BP	X						X							
Manifest refraction and near add	X													
[redacted]														
Biomicroscopy	X						X							
Corneal fluorescein staining and TBUT	X													
IOP[h]	X						X							
Ophthalmoscopy[i]	X						X							
AEs	X----------													----------X
Randomization[a]	X													
Subject questionnaire[l]	X*									X				
In-office Treatment 1: Nyxol or placebo[j]	X*													
Ocular tolerability[k]	X*						X							
Treatment 1: Nyxol or placebo and Subject diary dispensing/review[j]						X						X		

AE, adverse event; BCDVA, best-corrected distance visual acuity; BP, blood pressure; DCNVA, distance-corrected near visual acuity; HR, heart rate; ID, identification; IOP, intraocular pressure; OD, right eye; OS, left eye; PD, pupil diameter; VA, visual acuity.

[a] If the subject meets all the inclusion criteria and none of the exclusion criteria, this Screening Visit becomes the Baseline Visit. Visit 1 (Screening/Baseline)

assessments are recommended to be performed on the same day; however, in instances in which subjects are unable to complete the full day of assessments for Visit 1, subjects may be scheduled to return for the completion of Visit 1 within 1 week of Screening. If Visit 1 is split over 2 separate days, all Screening assessments up to and including randomization must occur on the first day, and then the subject should return to complete their post-randomization assessments on the second day. *Post-randomization assessments to be performed on the second day (ie, confirm concomitant medications, subject questionnaire, in-office Treatment 1 dosing, ocular tolerability, followed by all other assessments at time points 0.5, 1, 3, 5, and 8 hours post-treatment).
[b] Time of visits will be scheduled relative to the prior evening's dose of Treatment 1. Visit 2 will be scheduled such that the 0 hour (Time 0 min) assessments on Visit 2 will occur approximately 12 hours (± 1 hour) after the prior evening's dose of Treatment 1. Visit 3 occurs at Stage 1 Day 10 (±1 day) during the Washout Period.
[c] An additional ± 5 min is permitted for measurements at 0.5 and 1 hour; an additional ± 10 min is permitted at 3, 5, and 8 hours for Visits 1 and 2.
[d] Urine pregnancy test is for females of childbearing potential only.
[redacted]
[h] IOP performed with Tono-Pen or Goldmann applanation tonometry.
[i] Direct or indirect ophthalmoscopy performed without dilation. Use of 90 D lens (indirect) is allowed.
[j] Treatment 1: At Visit 1 (either first or second day of Visit 1, see * above), one drop of Nyxol or placebo (ie, Nyxol vehicle) will be dosed at the study site after the completion of 0 hour (Time 0 min) ocular assessments. Subject will dose with Treatment 1 each evening near bedtime, starting the evening of Stage 1 Day 2 through the evening prior to Visit 2 (Stage 1 Day 8), except the evening before Visit 2, when Treatment 1 is dosed as close to 12 hours prior to Time 0 min for that visit. Subjects will discontinue dosing during the 7- to 14-day Washout Period.
[k] Ocular tolerability performed [redacted]; it will be assessed at the beginning of each specified timepoint after dosing at Visits 1 and 2.
[l] Subject questionnaire (Appendix 5 and Appendix 6) will be completed by the subject at specified visits and timepoints.

Table 2: Schedule of Events for Stage 2

	Visit 4[a]							Visit 5[a]						
	Stage 2 Day 1 (Day 15 to 22)							Stage 2 Day 8 (7 days after Stage 2 Day 1)						
Time (hr)[b]	**12 hr post Treatment 1**	**0**	**0.5**	**1**	**3**	**5**	**8**	**12 hr post Treatment 1**	**0**	**0.5**	**1**	**3**	**5**	**8**
Prior/Concomitant medications	X							X						
Urine pregnancy test[c]	X							X						
HR/BP														X
[redacted]														
[redacted]														
[redacted]														
[redacted]														
[redacted]														
[redacted]														
[redacted]														
Biomicroscopy														X
IOP[g]														X
Ophthalmoscopy[h]														X
AEs	X----------													----------X
Subject questionnaire[k]					X							X		
In-office Treatment 2: LDP or LDP vehicle[i]		X							X					
Ocular tolerability[j]		X							X					
Treatment 1: Nyxol or placebo and Subject diary dispensed / reviewed[l]							X							X
Treatment 2: LDP or LDP vehicle and Subject diary dispensed / collected[i]							X							X

AE, adverse event; BCDVA, best-corrected distance visual acuity; BP, blood pressure; DCNVA, distance-corrected near visual acuity; HR, heart rate; ID, identification; IOP, intraocular pressure; LDP, Low-Dose Pilocarpine; OD, right eye; OS, left eye; PD, pupil diameter; VA, visual acuity.

[a] Time of visits will be scheduled relative to the prior evening's dose of Treatment 1. Visits 4 and 5 will be scheduled such that the 12 hour post-dose assessments on Visits 4 and 5 will occur approximately 12 hours (± 1 hour) after the prior evening's dose of Treatment 1.

[b] An additional ± 5 min is permitted for measurements at 0.5 and 1 hour; an additional ± 10 min is permitted at 3, 5, and 8 hours for Visits 4 and 5.

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

[redacted]

[redacted]

[g] IOP performed with Tono-Pen or Goldmann applanation tonometry.
[h] Direct or indirect ophthalmoscopy performed without dilation. Use of 90 D lens (indirect) is allowed.
[i] Treatment 2: One drop of LDP or LDP vehicle in each eye dosed daily in the mornings beginning on the day after Visit 4 and daily through the day prior to Visit 5. At Visits 4 and 5, subject will be dosed with Treatment 2 at the study site. Subject will bring Treatment 2 kit and diary to Visits 4 and 5 for review and accountability.
[j] Ocular tolerability performed [redacted] it will be assessed at the beginning of each specified timepoint after dosing at Visits 4 and 5.
[k] Subject questionnaire (Appendix 5 and Appendix 6) will be completed by the subject at specified visits and timepoints.
[l] Treatment 1: One drop of Nyxol or placebo in each eye dosed daily in the evenings near bedtime, except the evening before Visits 4 and 5, when Treatment 1 is dosed as close to 12 hours prior to the scheduled time for those visits. Subjects will bring their Treatment 1 kit and diary to Visits 4 and 5 for review and accountability.

5.3. Measures Taken to Minimize/Avoid Bias

This is a placebo-controlled, double-masked, randomized, 4-arm Phase 3 study.

5.4. Study Medications

5.4.1. 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 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 approximately 178ºC.

The properties of phentolamine mesylate are described in Table 3.

Table 3: Phentolamine Mesylate 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 (POS 0.75%) is a clear, colorless to slightly brown, sterile, non-preserved, isotonic, buffered aqueous solution containing 1% phentolamine mesylate (equivalent to 0.75% phentolamine free base), mannitol, sodium acetate, and water for injection. Placebo for Nyxol is a clear, colorless, sterile, non-preserved, isotonic, buffered aqueous solution containing mannitol, sodium acetate, and water for injection. The pH of both Nyxol and its placebo [redacted]

For the proposed study, a second treatment medication, Pilocarpine Hydrochloride Ophthalmic Solution 0.4% (LDP) will be used. Pilocarpine Hydrochloride Ophthalmic Solutions 1%, 1.25%, 2%, and 4% are approved by the US Food and Drug Administration (FDA) and available generically by multiple pharmaceutical manufacturers, including Sandoz Inc., Somerset LLC, Akorn Inc, and Alcon Laboratories.

Pilocarpine hydrochloride is the hydrochloride salt of (+)-pilocarpine (parent compound), a medication used to treat increased IOP, dry mouth, and presbyopia. 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 [redacted] The parent (+)-pilocarpine is a natural alkaloid extracted from plants of the genus *Pilocarpus* with cholinergic agonist activity.

The properties of PCH are described in Table 4.

Table 4: Pilocarpine Hydrochloride 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	Low-Dose Pilocarpine Hydrochloride Ophthalmic Solution 0.4% (LDP) 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	[redacted]
Storage requirements	[redacted] Stored in a secured location (locked) with no access for unauthorized personnel

Pilocarpine hydrochloride 0.4% investigational drug is compounded in pharmacy, starting with pilocarpine hydrochloride 4% produced by Sandoz, Inc. 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 [redacted]

Low-Dose Pilocarpine is primary-packaged in multi-use, low-density polyethylene (LDPE) dropper bottles for investigational use by subjects, and it is stored at room temperature (15°C to 25°C, 59°F to 77°F).

5.4.2. Packaging and Labeling

Nyxol and its placebo (Treatment 1) are packaged in a 0.5-mL LDPE Blow-Fill-Seal vial containing 0.3 mL solution for single-dose use. Five individual vials are included in a molded strip. Each strip containing 5 vials is wrapped with a multicolor "rainbow" aluminum foil overwrap that has been purged with nitrogen. The foil is impermeable to water and oxygen and will be labeled with an investigational label showing the study protocol number and other relevant information, including a statement "Caution – New Drug – Limited by Federal (US) Law to Investigational Use".

Low-Dose Pilocarpine and its placebo (Treatment 2) is provided in 15 mL LDPE, multi-use dropper bottles. They will be labeled with an investigational label showing the study protocol number and other relevant information, including a statement "Caution – New Drug – Limited by Federal (US) Law to Investigational Use."

5.4.3. 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 its placebo) should be shipped and stored at the study site [redacted]

Treatment 2 study medication (LDP and its placebo) should be stored [redacted]

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

5.4.4. Study Medication Administration

Subjects will restart dosing daily with Treatment 1 in the evening immediately prior to Visit 4 (Stage 2 Day 1). The evening doses prior to Visit 4 and Visit 5 (Stage 2 Day 8) should be as close to 12 hours prior to the scheduled time for those visits. At both of these visits, after completion of the 12-hour assessments for Treatment 1, subjects will be administered their morning dose of Treatment 2 (at Time 0 min) at the study site, and efficacy and safety measurements will be made at multiple post-dose timepoints over the next 8 hours.

5.4.5. Study Medication Accountability

5.4.5.1. Receipt and Disposition of Study Medication

The Investigator or designee (eg, 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 and subject diaries using the inventories supplied by Ocuphire. The Investigator or designee will account for both Treatment 1 and Treatment 2 study medications and subject diaries. The monitor will review dispensing and study medication accountability records during site visits and at the completion of the study and note any discrepancies.

5.4.5.2. 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 and subject diaries disposition will be completed by the study coordinator.

5.5. Expected Duration of Subject Participation

The total length of subject participation is approximately 22 to 36 days (accommodating for weekends), with 5 clinic visits as summarized below:

- Screening/Baseline Visit 1 (Stage 1 Day 1). Note that Visit 1 (Screening/Baseline) assessments are recommended to be performed on the same day; however, in instances in which subjects are unable to complete the full day of assessments for Visit 1, subjects may be scheduled to return for the completion of Visit 1 within 1 week of Screening. If Visit 1 is split over 2 separate days, all Screening assessments up to and including randomization must occur on the first day, and then the subject should return to complete their post-randomization assessments on the second day.
- Visit 2 (Stage 1 Day 8)
- Visit 3 (Stage 1 Day 10 ± 1 day) during the Washout Period of 7 to 14 days
- Visit 4 (Stage 2 Day 1 [Day 15-22])
- Visit 5 (Stage 2 Day 8 [Day 22-29])

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

5.6. Randomization and Procedure for Breaking the Code

A randomization code for allocating subjects to each treatment group will be prepared by an unmasked biostatistician not connected with the study. Subjects will be then randomized 1:1:1:1 into 1 of 4 treatment arms at Visit 1:

- Nyxol + LDP
- Nyxol + LDP vehicle
- Placebo + LDP
- Placebo + LDP vehicle

[redacted]

5.7. 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 will be verified electronically using a series of online programmed edit checks that have been created by the Clinical Data Manager and programmed by the Clinical Data Programmer or designee. Data discrepancies will be brought to the attention of the clinical team and investigated by the Study Monitor and Site Staff. Study Monitors will review and verify all data collected in the 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.

5.8. Completed Subject

A completed subject is defined as one who completes all planned procedures through the end of Visit 5.

5.9. 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.

5.9.1. Study Medication Discontinuation

The study medication may be discontinued for the following reasons:

- **Adverse events:** Adverse events include clinically significant laboratory abnormalities and intercurrent diseases reported by the subject or observed by the Investigator, with documentation on the eCRF
- **Death:** If a subject dies, the AE(s) that caused the death should be documented on the eCRF and be noted as serious and fatal

- **Disallowed concurrent medication:** Any medication not allowed by the protocol would be a protocol violation
- **Lack of efficacy:** A subject may elect to discontinue participation in the study for a perceived lack of efficacy
- **Investigator decision:** A subject may be discontinued for reasons other than those bulleted previously if the Investigator thinks it is not in the best interest of the subject to continue
- **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

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

5.9.2. Reasons for Withdrawal From Study

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

5.9.3. 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.

5.9.4. 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 8-hour measurements are assessed prior to discontinuation, in addition to a follow-up telephone call that includes assessments for AEs and concomitant medications.

5.10. 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 have been 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 are resolved.

5.11. Procedure After the Completion of the Study

When the study is completed, the contract research organization (CRO) will provide Ocuphire and the Investigator with a brief (ie, 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.

6. SUBJECT INCLUSION AND EXCLUSION CRITERIA

6.1. 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.

[redacted]

6.2. 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 (including Vuity) 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 (eg, OCuSOFT® lid scrub, SteriLid®, baby shampoo, etc.) and artificial tears as specified in Exclusion #2 below.
2. Use of any OTC artificial tears (preserved or unpreserved) during Visit days or 15 min before or after instillation of Treatment 1 or Treatment 2.
3. Current use of any dry eye product such as topical ophthalmic therapy for dry eye (eg, generic cyclosporine, Restasis, Xiidra, Cequa, and Eysuvis) or intranasal dry eye product (eg, Tyrvaya) or other devices.
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 (eg, cataract, glaucoma, corneal edema, uveitis, retinal degeneration, loss of visual field, or 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. Known allergy, hypersensitivity, or contraindication to any component of the phentolamine, pilocarpine, or vehicle formulations.
9. Prior participation in a study involving the use of Nyxol for the treatment of presbyopia.
10. History of cauterization of the punctum or punctal plug (silicone or collagen) insertion or removal.
11. Ocular trauma within 6 months prior to Screening.
12. Ocular surgery or any ocular laser treatment within 6 months prior to Screening.
13. Subjects with surgical monovision, multifocal or extended depth of focus IOLs are excluded (monofocal IOLs are accepted if in place > 6 months prior to Screening).
14. History of any traumatic (surgical or nonsurgical) or nontraumatic condition affecting the pupil or iris (eg, irregularly shaped pupil, neurogenic pupil disorder, iris atrophy, iridotomy, iridectomy, iritis, etc.).
15. Contact lens wear on the day of any study visit, and contact lenses must be removed for home dosing and for at least 10 minutes following dosing.

Systemic:

16. Known hypersensitivity or contraindication to alpha- and/or beta-adrenoceptor antagonists (eg, chronic obstructive pulmonary disease or bronchial asthma; abnormally low BP or HR; second- or third-degree heart blockage or congestive heart failure).
17. Known hypersensitivity or contraindication to any systemic cholinergic parasympathomimetic agent.
18. Clinically significant systemic disease (eg, uncontrolled diabetes, myasthenia gravis, cancer, hepatic, renal, endocrine, or cardiovascular disorders) that might interfere with the study as deemed by the judgment of the Investigator.
19. 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; however, Flomax (tamsulosin) is specifically excluded.
20. Participation in any investigational study within 30 days prior to Screening.
21. 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).

22. Resting HR outside the range of 50 to 110 beats per minute following at least a 5-min rest period in the sitting position at Visit 1 (Screening/Baseline). Heart rate may be repeated **only once** if outside the specified range, following another 5-min rest period in the sitting position.
23. Hypertension with resting diastolic BP > 105 mmHg or systolic BP > 160 mmHg following at least a 5-min rest period in the sitting position at Visit 1 (Screening/Baseline). Blood pressure may be repeated **only once** if outside the specified range, following another 5-min rest period in the sitting position.

7. TREATMENT OF SUBJECTS

Approximately 320 eligible subjects will be randomized into 1 of 4 treatment arms:

- Nyxol + LDP
- Nyxol + LDP vehicle
- Placebo + LDP
- Placebo + LDP vehicle

Subjects will be randomized 1:1:1:1 into the above groups. Randomization will be stratified by iris color (light/dark irides). The number of subjects in each irides type should be as close as possible to 1:1, with a minimum of 40% within each stratum.

7.1. Treatment Adherence

Following the completion of Visit 1 (Stage 1 Day 1), subjects will be dispensed Treatment 1 study drug for dosing at home in the evenings and a Treatment 1 subject diary to document dosing.

At the end of Visit 4 (Stage 2 Day 1), subjects will be dispensed Treatment 2 study drug for dosing at home in the mornings and a Treatment 2 subject diary to document dosing.

Subjects must bring their used and unused study medications and subject diaries with them to Visit 2 (Stage 1 Day 8), Visit 4 (Stage 2 Day 1), and Visit 5 (Stage 2 Day 8).

All subjects must be instructed on the importance of following the once-daily dosing regimen for each Treatment 1 and Treatment 2 and maintaining their dosing diaries.

Treatment adherence will be measured by counting the used and unused vials at each study visit and by reviewing dosing in the subject diaries for both Treatments 1 and 2.

7.2. Concomitant Medications

As noted in the exclusion criteria (Section 6.2), use of any topical prescription or OTC ophthalmic medications of any kind within 7 days of Screening is prohibited, with the exception of lid scrubs with OTC products (eg, OCuSOFT® lid scrub, SteriLid®, baby shampoo, etc.) and artificial tears, except the use of any OTC artificial tears (preserved or unpreserved) during Visit days or 15 min before or after instillation of Treatment 1 or Treatment 2.

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. However, Flomax (tamsulosin) is specifically excluded

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 products (eg, Contac®, Cosopt®) and non-combination products, the generic name is desired. The use of routine ophthalmic diagnostic pharmaceutical agents (eg, fluorescein and local anesthetic) will be allowed and should be documented. Any change in dosing parameters should also be recorded in the eCRF.

8. ASSESSMENT OF EFFICACY

8.1. Specification of the Efficacy Parameters

The primary efficacy endpoint is the percent of subjects with ≥ 15 letters of improvement in photopic binocular DCNVA and with < 5 letters of loss in photopic binocular BCDVA at 30 min post-LDP/vehicle comparing Nyxol + LDP to placebo + LDP vehicle at Visit 5 (Stage 2 Day 8). This comparison will be analyzed first using a hierarchical testing method.

Key secondary efficacy endpoints can be found in Section 5.1.

[redacted].

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

8.2. Assessing, Recording, and Analyzing of Efficacy Parameters

Visual acuity and PD assessments will be measured as described in Section 5.1.

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.

The timing for recording efficacy parameters may be found in Table 1 and Table 2.

8.2.1. Screening/Baseline Visit 1 (Stage 1 Day 1)

Individuals who are potential subjects will be identified by the study center to schedule the Screening Visit. If a subject successfully completes their Screening Assessments, then Visit 1 becomes the Baseline Visit. Note that Visit 1 (Screening/Baseline) assessments are recommended to be performed on the same day; however, in instances in which subjects are unable to complete the full day of assessments for Visit 1, subjects may be scheduled to return

for the completion of Visit 1 within 1 week of Screening. If Visit 1 is split over 2 separate days, all Screening assessments up to and including randomization must occur on the first day, and then the subject should return to complete their post-randomization assessments on the second day.

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 (ICF) is signed, and a subject number is assigned.

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

The subject will then undergo manifest refraction and near add, and several VA measurements, including [redacted]

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

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

At Visit 1 (Stage 1 Day 1), eligible subjects will be randomized, and the first dose of Treatment 1 will be administered at the study site at Time 0 min. Efficacy and safety measurements are made before dosing with Treatment 1 (Time 0 min) and at multiple post-dose timepoints from 30 min to 8 hours. If Visit 1 is split over 2 separate days, all Screening assessments up to and including randomization must occur on the first day, and then the subject should return to complete their post-randomization assessments in the following order on the second day (ie, confirm concomitant medications, subject questionnaire, in-office Treatment 1 dosing, ocular tolerability, followed by all other assessments at time points 0.5, 1, 3, 5, and 8 hours post-treatment). The following assessments are conducted during Visit 1:

Prior/Concomitant medication

Heart rate and BP

Pupil diameter (photopic and mesopic)

Distance-corrected near VA [redacted] BCDVA [redacted] DCIVA [redacted]); all VA measurements will be made [redacted], except selected measurements at 0 min [redacted]), which will be measured binocularly followed by OD and OS measurements

Biomicroscopy

Intraocular pressure

Ophthalmoscopy

Ocular tolerability

Subject questionnaire

Adverse events

Treatment 1/Diary dispensing

8.2.2. Visit 2 (Stage 1 Day 8)

At Visit 2 (Stage 1 Day 8), efficacy and safety measurements will be made at 12 hours after the dose of Treatment 1 study medication (12-hour endpoint):

Heart rate and BP

Urine pregnancy test (females of childbearing potential)

Concomitant medications

Pupil diameter (photopic and mesopic)

Distance-corrected near VA [redacted] BCDVA [redacted], DCIVA [redacted]); all VA measurements will be made binocularly, except selected measurements at 0 min [redacted] and 8 hours [redacted] which will be measured binocularly followed by OD and OS measurements

Biomicroscopy

Intraocular pressure

Ophthalmoscopy

Ocular tolerability

Subject questionnaire

Adverse events

After completion of these assessments, the subject will take no study drug for the next 7 to 14 days (Washout Period), after which Stage 1 is complete.

8.2.3. Visit 3 (Stage 1 Day 10 ± 1 day) – During Washout Period

Measurements will be made on Visit 3 (Stage 1 Day 10 ± 1 day) during the Washout Period to assess the absence of residual treatment effects:

Concomitant medications

[redacted]

Distance-corrected near VA ([redacted], BCDVA [redacted]

Adverse events

At the conclusion of the Washout Visit measurements, Stage 1 of the study is complete.

8.2.4. Visit 4 (Stage 2 Day 1 [Day 15-22])

Visit 4 occurs after completion of the Washout Period. During Visit 4, efficacy and safety measurements will be made approximately 12 hours after the dose of Treatment 1 study medication and at multiple timepoints after the dose of Treatment 2 (at Time 0 min):

Urine pregnancy test (females of childbearing potential)

Concomitant medications

[redacted]

Distance-corrected near VA (photopic and mesopic), BCDVA ([redacted]), DCIVA [redacted]). All VA measurements will be made [redacted]

Ocular tolerability

Subject questionnaire

Adverse events

Treatment 2/Diary dispensing

8.2.5. Visit 5 (Stage 2 Day 8 [Day 22-29])

Visit 5 occurs 7 days after Visit 4. During Visit 5, efficacy and safety measurements will be made approximately 12 hours after the dose of Treatment 1 study medication and at multiple timepoints after the dose of Treatment 2 (at Time 0 min) [note 30 minute measurements are key secondary endpoints]:

Heart rate and BP

Urine pregnancy test (females of childbearing potential)

Concomitant medications

Pupil diameter (photopic and mesopic)

Distance-corrected near VA ([redacted] BCDVA [redacted] DCIVA ([redacted]. All VA measurements will be made binocularly except for 0.5 hours (DCNVA [redacted] and 8 hours (DCNVA [redacted] BCDVA [redacted] which will be measured binocularly followed by OD and OS measurements

Biomicroscopy

Intraocular pressure

Ophthalmoscopy

Ocular tolerability

Subject questionnaire

Adverse events

Collection of used/unused Treatment 1 and Treatment 2 and subject diaries

8.2.6. Unscheduled Visits

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

As noted in Section 5.9.4, 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 and concomitant medications.

8.3. Visit Variation

Visit 3 may occur 1 to 3 days after Visit 2; Visit 4 may occur 8 to 15 days after completion of Visit 1; and Visit 5 may occur 22 to 36 days after Visit 1.

9. ASSESSMENT OF SAFETY

9.1. Specification of Safety Parameters

The primary safety measures are subject-evaluated ocular tolerability and AEs. Other safety measures include biomicroscopy, ophthalmoscopy, BCDVA, IOP, subject questionnaire, and systemic safety (as measured by HR and BP). Urine pregnancy tests for females of childbearing potential will also be conducted.

The assessment of safety will be evaluated by:

- Ocular tolerability [redacted]
- Biomicroscopy of the anterior segment including evaluation of lids, lashes, cornea, conjunctiva, iris, lens, and anterior chamber. Following topical fluorescein administration, the integrity of the cornea should be evaluated by fluorescein staining
- Ophthalmoscopy (direct or indirect) without dilation to evaluate the vitreous and posterior pole
- Best-corrected distance VA
- Intraocular pressure performed with a Tono-Pen or Goldmann Applanation tonometry
- Subject questionnaire
- Heart rate and BP (as per the site's normal equipment and procedures)
- Adverse events

9.2. Assessing, Recording, and Analyzing Safety Parameters

The timing for recording safety parameters may be found in Table 1 and Table 2.

9.3. 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 9.3.1).

All AEs/adverse reactions occurring during the study (ie, once the subject has signed the ICF) **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.

9.3.1. 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 temporally 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?" Adverse events 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 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 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. Investigators are cautioned to use the appropriate verbatim term on the AE form to describe this observation:

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

Expedited reporting of serious and unexpected adverse events: All SAEs (related and unrelated) will be recorded following subject signature of the ICF consent and until the completion of Visit 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 [redacted]. The Investigator must assess the SAE relationship and complete the SAE eCRF. The CRO may request additional information. Follow-up information (eg, discharge summary) will be retained in the subject's chart and a copy will be emailed to [redacted]. In addition, all SAEs should be recorded on the AE eCRF page with the serious question marked "Yes."

It is the Investigator's responsibility to notify the approving IRB of any SAEs on a timely basis, as instructed by 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 that is not reasonably associated with study medication administration does not require completion of the SAE form.

9.3.2. 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.

10. STATISTICS

10.1. Sample Size

Subjects will be randomized 1:1:1:1 to Nyxol + LDP, Nyxol + LDP vehicle, Placebo + LDP, and Placebo + LDP vehicle respectively. As a result, subjects will be randomized 1:1 to Treatment 1 (Nyxol or placebo).

An additional 20 subjects will be enrolled to account for dropouts, for a total of approximately 320 subjects to be randomized.

A diagram outlining the study design can be found in Appendix 4.

10.2. Analysis Populations

Modified Intention-to-Treat Population: The mITT Population will include all randomized subjects who received at least 1 drop of Treatment 1. The mITT Population will be used to analyze the primary endpoint as well as other efficacy endpoints.

Per Protocol Stage 1 Population: The PP Stage 1 (PPS1) Population will include all subjects in the mITT Population who receive 1 drop of Treatment 1 the day prior to Visit 2, have binocular DCNVA and BCDVA in photopic conditions at Baseline (Visit 1) and at 12 hours post-dose at Visit 2, and have no major protocol deviations considered to have significant impact on treatment outcome in Stage 1. The PPS1 Population will be used for analysis of select efficacy endpoints as specified in the SAP.

Per Protocol Stage 2 Population: The PP Stage 2 (PPS2) Population will include all subjects in the PPS1 Population who receive 1 drop of Treatment 1 the day prior to Visit 4, receive 1 drop of Treatment 2 at Visit 4, have binocular DCNVA and BCDVA in photopic conditions for at least 1 timepoint at Visit 4 after Treatment 2 dosing, and have no major protocol deviations considered to have significant impact on treatment outcome in Stage 2. The PPS2 Population will be used for analysis of select efficacy endpoints as specified in the SAP.

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 1 drop of study treatment (Treatment 1 or Treatment 2). The SP will be used to summarize safety variables.

Per Protocol Populations include PPS1 and PPS2 throughout this document.

10.3. Statistical Methods

10.3.1. General Considerations

[redacted]

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

[redacted]

10.3.2. 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 Populations, the ARP, and the SP. These data will also be provided in by-subject listings.

10.3.3. Subject Disposition

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

10.3.4. Medical History and Prior/Concomitant Medications

Medical history will be coded using the latest version of Medical Dictionary for Regulatory Activities (MedDRA, Version 25 or higher) 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 (March 2022 or later edition available at study start) 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.

10.3.5. Analysis of Efficacy

Efficacy will be assessed using the mITT and PP Populations, with subjects included in the treatment arm into which they were randomized. For the analysis of the primary efficacy endpoint, appropriate imputation techniques will be performed for missing observations if applicable; details will be provided in the study SAP. The primary comparison of interest is Stage 2 Nyxol + LDP versus placebo + LDP vehicle for the mITT 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 latest values taken prior to administration of Treatment 1, which will usually be the screening assessment values on Visit 1.

All efficacy data will be summarized by treatment group, study visit, and timepoint, as appropriate.

The primary efficacy endpoint is the percent of subjects with ≥ 15 letters of improvement in photopic binocular DCNVA and with < 5 letters of loss in photopic binocular BCDVA at 30 min post-LDP/vehicle comparing Nyxol + LDP to placebo + LDP vehicle at Visit 5 (Stage 2 Day 8).

The primary efficacy endpoint will be [redacted] as a covariate. 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 light/dark irides factor.

Secondary efficacy endpoints are indicated in Section 5.1. Each of the continuous secondary efficacy endpoints will be analyzed using analysis of covariance (ANCOVA) with change from Baseline as the dependent variable, treatment [redacted], 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, DCIVA, and BCDVA, will be analyzed using ETDRS letters correctly read.

For each of the secondary endpoints [redacted] 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 light/dark irides 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.

Select efficacy endpoints will be included [redacted], which will be specified in the SAP. The first endpoint in the hierarchy will be the primary efficacy endpoint, followed by key secondary endpoints.

10.3.6. 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.

For HR and BP, Baseline is defined as the Screening value. Heart rate and BP values and change from Baseline in the values will be summarized by treatment arm and select timepoints.

For IOP, Baseline is defined as the Screening value. Observed values and change from Baseline in IOP at select timepoints will be summarized by treatment arm for the study eye and the fellow eye.

For BCDVA, the number of subjects who lose ≥ 5 letters from Baseline will be summarized by treatment arm and select timepoints.

Ocular tolerability values will be summarized by treatment arm at select timepoints.

Verbatim descriptions of AEs will be coded using MedDRA. Only TEAEs (those that occur after the first dose of study medication *or increasing in severity after initiation of study medication*) will be summarized. Treatment-emergent AEs and SAEs will be summarized by treatment 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.

10.4. 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 SAP. For the summarization of safety data, observed case data only will be used.

10.5. Procedure for Reporting Deviations From the Statistical Plan

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

11. DIRECT ACCESS TO SOURCE DATA AND DOCUMENTS

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

12. QUALITY CONTROL AND QUALITY ASSURANCE

The progress of the study will be monitored by onsite, written, and telephone communications between personnel at the Investigator's site and the Medical Monitor. Should the 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 ICFs, records of study medication receipt, storage, preparation, disposition, and regulatory files related to this study.

13. ETHICAL CONSIDERATIONS AND GOOD CLINICAL PRACTICE COMPLIANCE

13.1. Good Clinical Practice Compliance

The proposed study is subject to all applicable governmental rules and regulations concerning the conduct of clinical trials on human subjects. This includes, but is not necessarily limited to, the approval of IRBs, the Helsinki Declaration, US FDA law, International Council for Harmonisation (ICH) 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.

13.2. Institutional Review Board

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

13.3. Protocol Deviations/Violations

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

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

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

13.4. Informed Consent Requirements

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

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

It should be emphasized that the subject is at liberty to withdraw consent to participate at any time, without penalty or loss of benefits to which the subject is otherwise entitled. Subjects who refuse to give or 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 ICF.

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

14. DATA HANDLING AND RECORD KEEPING

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

14.1. Data Entry

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

14.2. Data Quality Control and Reporting

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

14.3. Archiving of Data

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

Investigator when documents should be returned.

14.4. Records Retention

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

14.5. Amendments to the Protocol

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

The Investigator must not implement any deviation from or change to the protocol, without discussion with, and agreement by 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 (eg, 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).

15. REFERENCES

1. McDonald MB, Pitlick W, D.R. V, Meyer AR, E.D. D. Phentolamine Mesylate Treatment of Severe Night Vision Complaints. AAO Abstracts, PO433. 2010.

2. Holladay JT, Meyer A, Pitlick B. Phentolamine Mesylate Ophthalmic Solution Once Daily Reduces Pupil Diameter and Improves Night Vision Disturbances. AAO Annual Meeting, PA025. 2018.

3. Pepose JS, Pitlick B, Meyer A, Jaber R, Charizanis K, Slonim C, et al. Phentolamine mesylate ophthalmic solution provides long lasting pupil modulation and improves visual acuity. Investigative Ophthalmology & Visual Science. 2020;61(7):5100-.

4. Leavitt JA, Wayman LL, Hodge DO, Brubaker RF. Pupillary response to four concentrations of pilocarpine in normal subjects: application to testing for Adie tonic pupil. Am J Ophthalmol. 2002;133(3):333-6.

5. Edgar DF, Crabb DP, Rudnicka AR, Lawrenson JG, Guttridge NM, O'Brien CJ. Effects of dipivefrin and pilocarpine on pupil diameter, automated perimetry and LogMAR acuity. Graefes Arch Clin Exp Ophthalmol. 1999;237(2):117-24.

6. AOA. American Optometric Association - Adult Vision: 41 to 60 Years of Age.

7. McDonnell PJ, Lee P, Spritzer K, Lindblad AS, Hays RD. Associations of presbyopia with vision-targeted health-related quality of life. Arch Ophthalmol. 2003;121(11):1577-81.

8. Fricke TR, Tahhan N, Resnikoff S, Papas E, Burnett A, Ho SM, et al. Global Prevalence of Presbyopia and Vision Impairment from Uncorrected Presbyopia: Systematic Review, Meta-analysis, and Modelling. Ophthalmology. 2018;125(10):1492-9.

9. Waring GO, Price FW, Wirta D, et al, Safety and Efficacy of AGN-190584 in Individuals with Presbyopia, The GEMINI 1 Phase 3 Randomized Clinical Trial, JAMA Ophthalmol. 2022;140(4):363-71.

10. Tucker J, Charman WN. The depth-of-focus of the human eye for Snellen letters. Am J Optom Physiol Opt. 1975;52(1):3-21.

11. Dexl AK, Seyeddain O, Riha W, Hohensinn M, Hitzl W, Grabner G. Reading performance after implantation of a small-aperture corneal inlay for the surgical correction of presbyopia: Two-year follow-up. J Cataract Refract Surg. 2011;37(3):525-31.

12. Xu R., Thibos L., Bradley A., Effect of Target Luminance on Optimum Pupil Diameter for Presbyopic Eyes. Optometry and Vision Science. 2016;91(11):1409-19.

13. Hogan TS, McDaniel DD, Bartlett JD, Hart KK, Paggiarino DA. Dose-response study of dapiprazole HCl in the reversal of mydriasis induced by 2.5% phenylephrine. J Ocul Pharmacol Ther. 1997;13(4):297-302.

16. SUMMARY OF CHANGES

Bold text shows additions; ~~strikethrough text~~ shows deletions. For changes that affect multiple sections of the protocol, the change is listed once at the first instance in the table below, and each subsequent protocol section incorporating that change is also listed at that point. Administrative and minor editing changes that do not affect the content or conduct of the protocol have been made; these are not listed.

16.1. Protocol OPI-NYXP-301 Amendment 1

Protocol OPI-NYXP-301 Amendment 1, issued 31 October 2022, makes the following changes to the original protocol dated 08 July 2022:

Table 5 Protocol OPI-NYXP-301 Amendment 1 Summary of Changes

Section/Location	Description of Change	Rationale for Change
Synopsis: Inclusion criteria (p.7) 6.1.Subject Inclusion Criteria (p.37)	**Inclusion criterion #8:** [redacted]	[redacted]
Synopsis: Exclusion criteria (p.8) 6.2.Subject Exclusion Criteria (p.38)	Exclusion criterion #15: **Contact lens wear on the day of any study visit and contact lenses must be removed for home dosing and for at least 10 minutes following dosing.**	To clarify that contact lens wearers must remove contact lenses prior to dosing and leave them out for at least 10 minutes after dosing
Synopsis: Key Secondary Efficacy Endpoints (p.10)	[redacted]	[redacted]

<table>
<tr><th>Section/Location</th><th>Description of Change</th><th>Rationale for Change</th></tr>
<tr><td>5.4.1 Study Medication: Table 4 (p.32-33)</td><td>
<table>
<tr><td>Investigational product name/formulation</td><td>Low-Dose Pilocarpine Hydrochloride Ophthalmic Solution 0.4% (LDP)
Aqueous isotonic solution, [redacted]</td></tr>
<tr><td>Concentration active</td><td>0.4%</td></tr>
<tr><td>Drug used in compounding the investigational product</td><td>PILOCARPINE HYDROCHLORIDE 4% - pilocarpine hydrochloride solution/ drops
NDC 61314-0206-15</td></tr>
<tr><td>Manufacturer drug product used for compounding</td><td>Sandoz, Inc.</td></tr>
</table>
~~Low-Dose Pilocarpine investigational drug is a sterile solution manufactured under current Good Manufacturing Practice (cGMP) conditions. In addition to the active drug substance, the formulation also comprises the following inactive excipients: benzalkonium chloride NF, hypromellose 2910 USP, sodium citrate USP, boric acid NF, sodium chloride USP, and USP purified water. 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 0.4% investigational drug is compounded in pharmacy, starting with pilocarpine hydrochloride 4% produced by Sandoz, Inc. 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.** [redacted]</td><td>[redacted]</td></tr>
<tr><td>5.1 Primary and Secondary Endpoints (p.26)</td><td>[redacted]</td><td>To provide the equivalence luminance levels used in lux</td></tr>
</table>

Appendix 3: Adrenergic and Cholinergic Drugs (p.63-64)	*Alpha-1-agonists* **Metaraminol** **Methoxamine** **Midodrine** **Amidephrine** *Alpha-2-agonists* **Norepinephrine** **Lofexidine** **Medetomidine** ***Undetermined alpha agonists*** **Ergotamine** **Etilefrine** **Indanidine** **Mephentermine** **Metaraminol** **Methoxamine** *Non-selective alpha-antagonists* **Trazodone** **Phentolamine** *Alpha-1-antagonists* **Silodosin** **Fuzosin** *Alpha-2-antagonists* **Raulscine** **Mirtazapine** ***Direct-acting acetylcholine receptor agonists*** ***Choline esters*** **Acetylcholine** **Bethanechol** **Carbachol** **Methacholine** ***Plant alkaloids*** **Arecoline** **Nicotine** **Muscarine** **Pilocarpine** ***Indirect-acting acetylcholine receptor agonists*** ***Reversible cholinesterase inhibitors*** **Donepezil** **Edrophonium** **Neostigmine** **Physostigmine** **Pyridostigmine** **Rivastigmine**	To make this list of adrenergic and cholinergic drugs more comprehensive

	Tacrine **Caffeine** **Huperzine A** ***Irreversible cholinesterase inhibitors*** **Echothiophate** **Isoflurophate** **Malathion** ***Antimuscarinic agents*** ***Antipsychotics (clozapine, quetiapine)*** **Atropine** **Benztropine** **Biperiden** **Chlorpheniramine** ***Certain SSRIs (Paroxetine)*** **Dicyclomine (Dicycloverine)** **Dimenhydrinate** **Diphenhydramine** **Doxepin** **Doxylamine** **Flavoxate** **Glycopyrrolate** **Glycopyrronium** **Hyoscyamine** **Ipratropium** **Orphenadrine** **Oxitropium** **Oxybutynin** **Promethazine** **Propantheline bromide** **Scopolamine** **Solifenacin** **Tolterodine** **Tiotropium** ***Tricyclic antidepressants*** **Trihexyphenidyl** **Tropicamide** **Umeclidinium** ***Antinicotinic agents*** **Bupropion** **Dextromethorphan** **Doxacurium** **Hexamethonium** **Mecamylamine** **Tubocurarine**	

16.2. Protocol OPI-NYXP-301 Amendment 2

Protocol OPI-NYXP-301 Amendment 2, issued 15 November 2022, makes the following changes to the Protocol OPI-NYXP-301 Amendment 1 dated 31 October 2022:

Table 6 Protocol OPI-NYXP-301 Amendment 2 Summary of Changes

Section/Location	Description of Change	Rationale for Change
Synopsis: Duration of Study (p.3) 5.5. Expected Duration of Subject Participation (p.35)	22 to ~~29~~**36** days	The change is to allow this visit to occur on 2 separate days so that the subject can return for dosing and Day 1 post-dose measurements. The rationale for this change is to allow for more flexible scheduling for the subject.
Synopsis: Design (p.4)	Subjects will be randomized 1:1:1:1 to treatments for both stages at Visit 1 (Screening/Baseline). **Note that Visit 1 (Screening/Baseline) assessments are recommended to be performed on the same day; however, in instances in which subjects are unable to complete the full day of assessments for Visit 1, subjects may be scheduled to return for the completion of Visit 1 within 1 week of Screening. If Visit 1 is split over 2 separate days, all visit assessments up to and including randomization must occur on the first day, and then the subject should return to complete their post-randomization assessments on the second day.** In Stage 1, the safety and efficacy of Nyxol as a single agent will be evaluated following 1 day and 7 days of daily dosing, in which Treatment 1 (Nyxol or placebo) is dosed daily in the evenings near bedtime **starting with the day after completion of Visit 1,** except for Visit 1 [Stage 1 Day 1], when the subject is dosed during the day at the study site, and the evening before Visit 2, when Treatment 1 is dosed as close to 12 hours prior to Time 0 min at Visit 2. During Stage 1, subjects will dose with Treatment 1 (Nyxol or placebo [ie, Nyxol vehicle]) in the evenings near bedtime **starting with the day after completion of Visit 1,** except for Visit 1 [Stage 1 Day 1], when the subject is dosed during the day at the study site, and the evening before Visit 2, when Treatment 1 is dosed as close to 12 hours prior to Time 0 min at Visit 2.	The change is to allow this visit to occur on 2 separate days so that the subject can return for dosing and Day 1 post-dose measurements. The rationale for this change is to allow for more flexible scheduling for the subject.
Synopsis: Design (p.5) 5. Study Design (p.24)	The study eye and fellow eye will both be evaluated at all assessments. **The baseline values are determined during Screening assessments at Visit 1.**	The change is to allow this visit to occur on 2 separate days so that the subject can return for dosing and Day 1 post-dose measurements. The rationale for this change is to allow for more flexible scheduling for the subject.

Section/Location	Description of Change	Rationale for Change
Synopsis: Design (p.5)	At Visit 1 (Screening/Baseline), eligible subjects will be randomized, and the first dose of Treatment 1 will be administered **(note: if Visit 1 is split into 2 separate days, then dosing occurs on the second day)**. Efficacy and safety measurements are made before dosing with Treatment 1 (Time 0 min) and at multiple post-dose timepoints from 30 min to 8 hours. Prior to discharge ~~on~~**following completion of** Visit 1 (Stage 1 Day 1), Treatment 1 study medication for Stages 1 and 2 will be dispensed for evening dosing by the subject before bedtime, to begin ~~starting~~ the night of Stage 1 Day 2.	The change is to allow this visit to occur on 2 separate days so that the subject can return for dosing and Day 1 post-dose measurements. The rationale for this change is to allow for more flexible scheduling for the subject.
Synopsis: Study Drug Dispensing and Treatment Adherence (p.6) 7.1. Treatment Adherence (p.40)	~~At~~**Following the completion of** Visit 1 (Stage 1 Day 1), subjects will be dispensed Treatment 1 study drug for dosing at home in the evenings and a Treatment 1 subject diary to document dosing.	The change is to allow this visit to occur on 2 separate days so that the subject can return for dosing and Day 1 post-dose measurements. The rationale for this change is to allow for more flexible scheduling for the subject.
3.5. Study Population (p.22)	At Visit 1 (Screening/Baseline), eligible subjects will be randomized, and the first dose of Treatment 1 will be administered **(note: if Visit 1 is split into 2 separate days, then dosing occurs on the second day)**. Efficacy and safety measurements are made before dosing with Treatment 1 (Time 0 min) and at multiple post-dose timepoints from 30 min to 8 hours. **If Visit 1 is split over 2 separate days, all Screening assessments up to and including randomization must occur on the first day, and then the subject should return to complete their post-randomization assessments in the following order on the second day (ie, confirm concomitant medications, subject questionnaire, in-office Treatment 1 dosing, ocular tolerability, followed by all other assessments at time points 0.5, 1, 3, 5, and 8 hours post-treatment).** Prior to discharge ~~on~~**following completion of** Visit 1 (Stage 1 Day 1), Treatment 1 study medication for Stages 1 and 2 will be dispensed for evening dosing by the subject before bedtime, to begin ~~starting~~the night of Stage 1 Day 2.	The change is to allow this visit to occur on 2 separate days so that the subject can return for dosing and Day 1 post-dose measurements. The rationale for this change is to allow for more flexible scheduling for the subject.
5. Study Design (p.24)	Baseline for the study is defined as pre-treatment **up to and including randomization** (prior to Time 0 min) at Visit 1 (Screening/Baseline).	The change is to allow this visit to occur on 2 separate days so that the subject can return for dosing and Day 1 post-dose measurements. The rationale for this change is to allow for more flexible scheduling for the subject.
Table 1: Schedule of Events for Stage 1 (p.28) Table 2: Schedule of Events for Stage 2 (p.30)	Moved “Subject questionnaire” row up to occur right before in-office Treatment 1 dosing	The change is to allow this visit to occur on 2 separate days so that the subject can return for dosing and Day 1 post-dose measurements. The rationale for this change is to allow for more flexible scheduling for the subject.

Section/Location	Description of Change	Rationale for Change
Table 1: Schedule of Events for Stage 1 (p.28-29)	[a] If the subject meets all the inclusion criteria and none of the exclusion criteria, this Screening Visit becomes the Baseline Visit. **Visit 1 (Screening/Baseline) assessments are recommended to be performed on the same day; however, in instances in which subjects are unable to complete the full day of assessments for Visit 1, subjects may be scheduled to return for the completion of Visit 1 within 1 week of Screening. If Visit 1 is split over 2 separate days, all Screening assessments up to and including randomization must occur on the first day, and then the subject should return to complete their post-randomization assessments on the second day. *Post-randomization assessments to be performed on the second day (ie, confirm concomitant medications, subject questionnaire, in-office Treatment 1 dosing, ocular tolerability, followed by all other assessments at time points 0.5, 1, 3, 5, and 8 hours post-treatment).**	The change is to allow this visit to occur on 2 separate days so that the subject can return for dosing and Day 1 post-dose measurements. The rationale for this change is to allow for more flexible scheduling for the subject.
Table 1: Schedule of Events for Stage 1 (p.29)	[j] Treatment 1: At Visit 1 **(either first or second day of Visit 1, see * above)**, one drop of Nyxol or placebo (ie, Nyxol vehicle) will be dosed at the study site after the completion of 0 hour (Time 0 min) ocular assessments.	The change is to allow this visit to occur on 2 separate days so that the subject can return for dosing and Day 1 post-dose measurements. The rationale for this change is to allow for more flexible scheduling for the subject.
5.5. Expected Duration of Subject Participation (p.35) 8.2.1. Screening/Baseline Visit 1 (Stage 1 Day 1) (p.41-42)	**Note that Visit 1 (Screening/Baseline) assessments are recommended to be performed on the same day; however, in instances in which subjects are unable to complete the full day of assessments for Visit 1, subjects may be scheduled to return for the completion of Visit 1 within 1 week of Screening. If Visit 1 is split over 2 separate days, all Screening assessments up to and including randomization must occur on the first day, and then the subject should return to complete their post-randomization assessments on the second day.**	The change is to allow this visit to occur on 2 separate days so that the subject can return for dosing and Day 1 post-dose measurements. The rationale for this change is to allow for more flexible scheduling for the subject.
8.2.1. Screening/Baseline Visit 1 (Stage 1 Day 1) (p.42)	Efficacy and safety measurements are made before dosing with Treatment 1 (Time 0 min) and at multiple post-dose timepoints from 30 min to 8 hours. **If Visit 1 is split over 2 separate days, all Screening assessments up to and including randomization must occur on the first day, and then the subject should return to complete their post-randomization assessments in the following order on the second day (ie, confirm concomitant medications, subject questionnaire, in-office Treatment 1 dosing, ocular tolerability, followed by all other assessments at time points 0.5, 1, 3, 5, and 8 hours post-treatment).**	The change is to allow this visit to occur on 2 separate days so that the subject can return for dosing and Day 1 post-dose measurements. The rationale for this change is to allow for more flexible scheduling for the subject.
8.3. Visit Variation (p.45)	Visit 3 may occur 1 to 3 days after Visit 2; Visit 4 may occur 8 to 15 ~~and~~ days after **completion of** Visit 1; and Visit 5 may occur 22 to ~~29~~**36** days after Visit 1.	The change is to allow this visit to occur on 2 separate days so that the subject can return for dosing and Day 1 post-dose measurements. The rationale for this change is to allow for more flexible scheduling for the subject.

16.3. Protocol OPI-NYXP-301 Amendment 3

Protocol OPI-NYXP-301 Amendment 3, issued 29 September 2023, makes the following changes to the Protocol OPI-NYXP-301 Amendment 2, issued 15 November 2022:

Table 7 Protocol OPI-NYXP-301 Amendment 3 Summary of Changes

Section/Location	Description of Change	Rationale for Change
Synopsis: Study Objectives (p.3) 4.Objectives and Purpose (p.23)	The primary objective of this study is: • To evaluate the efficacy of ~~Nyxol as a single agent to improve distance-corrected near visual acuity (DCNVA) without loss of best-corrected distance visual acuity (BCDVA) compared to placebo in subjects with presbyopia~~ **LDP as adjunctive therapy to Nyxol (Nyxol + LDP) to improve distance-corrected near visual acuity (DCNVA) without loss of best-corrected distance visual acuity (BCDVA) compared to placebo + LDP vehicle in subjects with presbyopia** Additional objectives of this study are: • ~~To evaluate the efficacy of LDP as adjunctive therapy to Nyxol (Nyxol + LDP) to improve DCNVA without loss of BCDVA compared to placebo, Nyxol alone, and LDP alone, in subjects with presbyopia~~ • **To evaluate the efficacy of Nyxol + LDP to improve DCNVA without loss of BCDVA compared to placebo + LDP (LDP alone) and Nyxol + LDP vehicle (Nyxol alone) in subjects with presbyopia** • **To evaluate the efficacy of Nyxol as a single agent to improve DCNVA without loss of BCDVA compared to placebo in subjects with presbyopia**	To move evaluation of Nyxol as a single agent from the primary objective to a secondary objective and to move evaluation of Nyxol + LDP from a secondary objective to the primary objective
Synopsis: Design (p.6) 3.5 Study Population (p.22) 8.2.2 Visit 2 (Stage 1 Day 8) (p.43)	At Visit 2 (Stage 1 Day 8), efficacy and safety measurements will be made at 12 hours after the dose of Treatment 1 study medication (12-hour ~~primary~~ endpoint).	
Synopsis: Primary Efficacy Endpoint (p.10) 5.1 Primary and Secondary Endpoints (p.25) 8.1 Specification of the Efficacy Parameters (p.41) 10.3.5 Analysis of Efficacy (p.52)	**The primary efficacy endpoint is the percent of subjects with ≥ 15 letters of improvement in photopic binocular DCNVA and with < 5 letters of loss in photopic binocular BCDVA at 30 min post-LDP/vehicle comparing Nyxol + LDP to placebo + LDP vehicle at Visit 5 (Stage 2 Day 8).** ~~The primary efficacy endpoint is the percent of subjects with ≥ 15 letters of improvement in photopic binocular DCNVA and with < 5 letters of loss in photopic binocular BCDVA from Baseline comparing Nyxol-treated subjects to placebo-treated subjects at 12 hours post-dose at Visit 2 (Stage 1 Day 8).~~	

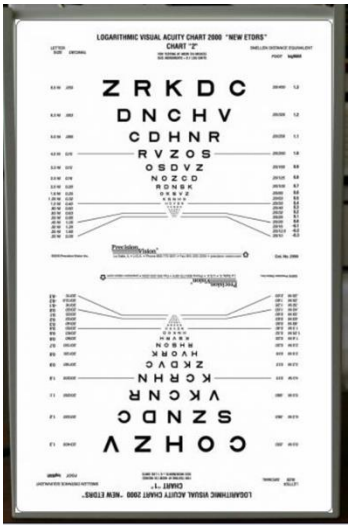
Section/Location	Description of Change	Rationale for Change
Synopsis: Key Secondary Efficacy Endpoints (p.10) 5.1 Primary and Secondary Endpoints (p.25)	[redacted]	
Synopsis: Other Secondary Efficacy Endpoints (p.10-11) 5.1 Primary and Secondary Endpoints (p.25)	• Percentage of subjects with ≥ 15 letters of improvement in binocular photopic DCNVA and with < 5 letters of loss in binocular photopic BCDVA from Baseline (excluding primary **and key secondary** outcome timepoints)	
10.3.5 Analysis of Efficacy (p.51)	The primary comparison of interest is Stage **2~~1~~** Nyxol **+ LDP versus**~~and~~ placebo **+ LDP vehicle** ~~alone~~ for the mITT Population.	
Synopsis: Other Secondary Efficacy Endpoints (p.11) 5.1 Primary and Secondary Endpoints (p.26)	• **Subject questionnaire responses related to change in near vision and satisfaction with near vision**	To add subject questionnaire responses as an efficacy endpoint

APPENDIX 1: IRIS COLOR CHART

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

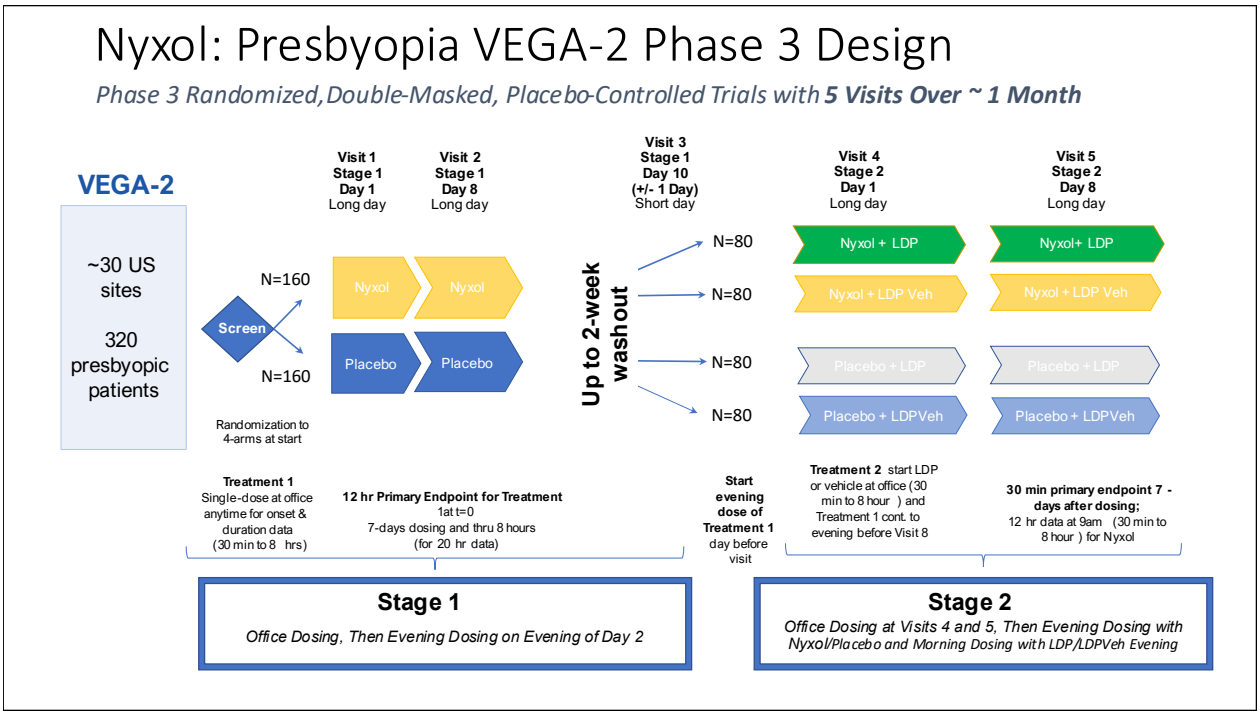
APPENDIX 3: ADRENERGIC AND CHOLINERGIC DRUGS

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; ***however, tamsulosin is specifically excluded***. *This list is not inclusive of all drugs in these classes. If there is any doubt, please consult with the Medical Monitor.*

Alpha-1-agonists Methyl norepinephrine Naphazoline Oxymetazoline Tetrahydrozoline Phenylephrine Xylometazoline Metaraminol Methoxamine Midodrine Amidephrine *Alpha-2-agonists* Brimonidine Clonidine Guanfacine Guanabenz Guanoxabenz Guanethidine Xylazine Tizanidine Methyldopa Norepinephrine Lofexidine Medetomidine *Undetermined alpha agonists* Ergotamine Etilefrine Indanidine Mephentermine Metaraminol Methoxamine	*Non-selective alpha-antagonists* Phenoxybenzamine Tolazoline Labetalol Carvedilol Trazodone Phentolamine *Alpha-1-antagonists* Alfuzosin Prazosin Doxazosin Tamsulosin Terazosin Silodosin Fuzosin *Alpha-2-antagonists* Atipamezole Idazoxan Yohimbine Raulscine Mirtazapine	***Direct-acting acetylcholine receptor agonists*** *Choline esters* Acetylcholine Bethanechol Carbachol Methacholine *Plant alkaloids* Arecoline Nicotine Muscarine Pilocarpine ***Indirect-acting acetylcholine receptor agonists*** *Reversible cholinesterase inhibitors* Donepezil Edrophonium Neostigmine Physostigmine Pyridostigmine Rivastigmine Tacrine Caffeine Huperzine A *Irreversible cholinesterase inhibitors* Echothiophate Isoflurophate Malathion *Acetylcholine receptor antagonists* Scopolamine	*Gastrointestinal* Atropine Belladonna *Parkinsonism* Amantadine Benztropine Biperiden Trihexyphenidyl ***Antimuscarinic agents*** *Antipsychotics (clozapine, quetiapine)* Atropine Benztropine Biperiden Chlorpheniramine *Certain SSRIs (Paroxetine)* Dicyclomine (Dicycloverine) Dimenhydrinate Diphenhydramine Doxepin Doxylamine Flavoxate Glycopyrrolate Glycopyrronium Hyoscyamine Ipratropium Orphenadrine Oxitropium Oxybutynin Promethazine Propantheline bromide

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

APPENDIX 4: STUDY DESIGN



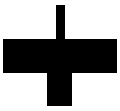
APPENDIX 5: SUBJECT QUESTIONNAIRE FOR BASELINE VISIT

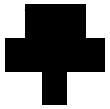
Subject Number: _____ Visit Date_____
Time: _____

VEGA-2 Subject Questionnaire – Baseline Visit

As you answer the subject questionnaire, please use the following examples as a reference:
Near vision: *seeing things up close, such as a cell phone or book*
Intermediate vision: *seeing things that are not handheld, but still close, such as the dashboard of a car or a desktop computer*
Distance vision: *seeing things far away, such as road signs while driving or a TV screen*
Using/doing something to manage near vision*: may include wearing reading or bifocal glasses, contact lenses, squinting, or adjusting font size or zooming in (when using screens)*

I. For **questions 1-6**, circle the answer most applicable to you, without something to manage your vision for **questions 4-6.**

1. What do you use most to help with managing your **near** vision?					
2. For how many hours in a typical day do you use or do something to help manage your **near** vision?					
3. How important is clear **near** vision from the time you wake up?					
4. How would you describe your **near** vision in normal or day light?					
5. How would you describe your **near** vision in dim or evening light?					

6. How difficult is it for you to see **<u>distance while driving</u>** in dim or night light?						

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II. For each of the **questions 7-12**, circle the number that corresponds with the frequency that is most applicable to you over the past week. If you have not used screens in the past week, please circle "N/A" for **questions 9** and **10**.

Over the **past week**…						
7. How frequently did you use some form of **<u>near</u>** vision correction?						
8. How often did you need to use or do something to help you read small-sized text at a **<u>near</u>** distance (e.g., a menu or prescription label)						
9. How often did you need to use or do something to help you read information on a cell phone or tablet screen at a **<u>near</u>** distance?						N/A
10. How often did you need to use or do something to help you read information on a computer screen at an **<u>intermediate</u>** distance?						N/A
11. How often did you need to use or do something to help you see things clearly from a **<u>distance</u>**?						
12. How often was your self-confidence negatively affected by the ways you manage your **<u>near</u>** vision?						

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APPENDIX 6: SUBJECT QUESTIONNAIRE FOR VISITS 2, 4, AND 5

Subject Number: _____ Visit #_____ Date _____ Time _____

VEGA-2 Subject Questionnaire – Visits 2, 4, and 5

As you answer the subject questionnaire, please use the following examples as a reference:

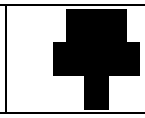
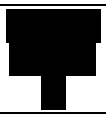
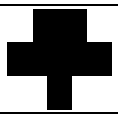
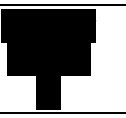
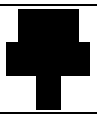
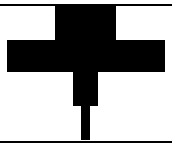
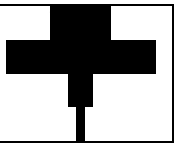
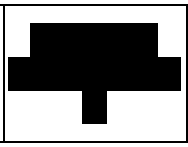
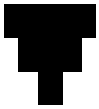
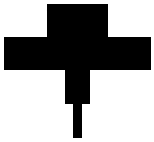
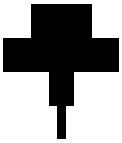
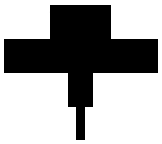
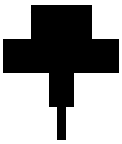
Near vision: *seeing things up close, such as a cell phone or book*

Intermediate vision: *seeing things that are not handheld, but still close, such as the dashboard of a car or a desktop computer*

Distance vision: *seeing things far away, such as road signs while driving or a TV screen*

Using/doing something to manage near vision*: may include wearing reading or bifocal glasses, contact lenses, squinting, or adjusting font size or zooming in (when using screens)*

I. For each of the **questions 1-8**, circle the answer that is most applicable to your **overall experience with your study medication**.

1. My study medication made my **near** vision…					
2. How convenient was it to use your study medication for your **near** vision?					
3. How **frequently** would you use the study medication if it were available to you?					
4. How satisfied are you with **how long the effects** of your study medication lasted throughout the day?					
5. How satisfied are you with your study medication to help you see **clearly up close** when you first wake up?					

6. My study medication made my **near** vision in dim/low light …					

7. My study medication made my **distance** vision…					

8. My study medication made my **distance** vision while driving at night…						

II. For **question 9**, please circle **every answer** that applies to you.

9. If the study medication were available to me, I would use it for…						

III. For each of the **questions 10-13**, circle the number that corresponds with the frequency that is most applicable to you over the past week. If you have not used screens in the past week, please circle "N/A" for **questions 12** and **13**.

Over the **past week**…						N/A
10. How frequently did you use some form of **near** vision correction?						
11. How often did you need to use or do something to help you read small-sized text at a **near** distance (e.g., a menu or prescription label)?						
12. How often did you need to use or do something to help you read information on a screen (e.g., a cell phone or tablet) at a **near** distance?						N/A
13. How often did you need to use or do something to help you read information on a screen at an **intermediate** distance?						N/A

IV. For **question 14**, circle the answer that is most applicable to you.

14. How likely would you be to use the study medication for **near** vision correction?