CONFIDENTIAL

Clinical Study Phase 3 Protocol
OPI-NYXRM-302
MIRA-3

Randomized, Parallel-Arm, Double-Masked, Placebo-Controlled Study of the Safety and Efficacy of Nyxol (0.75% Phentolamine Ophthalmic Solution) to Reverse Pharmacologically Induced Mydriasis in Healthy Subjects

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Version: **01**
Original: **23 September 2021**

SPONSOR SIGNATURE & CONTACTS

Study Title:	Randomized, Parallel-Arm, Double-Masked, Placebo-Controlled Study of the Safety and Efficacy of Nyxol (0.75% Phentolamine Ophthalmic Solution) to Reverse Pharmacologically Induced Mydriasis in Healthy Subjects
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Original Protocol:	Version 01; 23 September 2021

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

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INVESTIGATOR'S AGREEMENT

OPI-NYXRM-302
MIRA-3

Randomized, Parallel-Arm, Double-Masked, Placebo-Controlled Study of the Safety and Efficacy of Nyxol (0.75% Phentolamine Ophthalmic Solution) to Reverse Pharmacologically Induced Mydriasis in Healthy Subjects

Version: **01**

Original: **23 September 2021**

Investigator Agreement:

I, the undersigned, have reviewed this protocol and I agree to conduct this protocol in accordance with Good Clinical Practice, the ethical principles set forth in the Declaration of Helsinki and with the U.S. Code of Federal Regulations governing the protection of human subjects (21 CFR 50), Institutional Review Boards (21 CFR 56) and the obligations of clinical investigators (21 CFR 312).

Signature: _____ Date: _____

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PROCEDURES IN CASE OF EMERGENCY

EMERGENCY CONTACT INFORMATION

Role in Study	Name	Contact Information
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ABBREVIATIONS AND TERMS

Abbreviation	*Full term*
AE	adverse event
ANCOVA	analysis of covariance
ARP	All Randomized Population
BAT	Brightness Acuity Tester
BCDVA	best-corrected distance visual acuity
BP	blood pressure
°C	degree Centigrade
CCLRU	Cornea and Contact Lens Research Unit
CI	confidence interval
CRA	clinical research associate
CRO	clinical research organization
°F	degree Fahrenheit
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
IOP	intraocular pressure
IRB	Institutional Review Board
ITT	Intent-to-Treat
IUD	intrauterine device
LC-MS/MS	liquid chromatography-tandem mass spectrometry

LSM	least squares mean
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intent-to-Treat
Nyxol	0.75% Phentolamine Ophthalmic Solution or 1% Phentolamine Mesylate Ophthalmic Solution (Nyxol®)
OD	oculus dexter (right eye)
OR	odds ratio
OS	oculus sinister (left eye)
OTC	over-the-counter
OU	oculus uterque (both eyes)
Paremyd®	1% hydroxyamphetamine hydrobromide/0.25% tropicamide
PD	pupil diameter
PK	pharmacokinetic
PP	Per Protocol
RAF	Royal Air Force
SAE	serious adverse event
SOC	system organ class
SP	Safety Population
TEAE	treatment-emergent adverse event
US	United States
USP	United States Pharmacopeia

1. STUDY SUMMARY

Study Number	**OPI-NYXRM-302**
Clinical Phase	Phase 3
Type of Study	Randomized, parallel-arm, double-masked, placebo-controlled study of the safety and efficacy of Nyxol (0.75% Phentolamine Ophthalmic Solution) to reverse pharmacologically induced mydriasis in healthy subjects
Name of Investigational Product	Nyxol® Eye Drops – 0.75% Phentolamine Ophthalmic Solution
Duration of Study	[redacted] including screening/treatment and follow-up
Rationale	Nyxol is a once-daily preservative-free eye drop formulation of phentolamine, which is a non-selective alpha-1 and alpha-2 adrenergic antagonist that acts on the adrenergic receptors and inhibits contraction of the smooth muscle. Phentolamine inhibits contraction of the iris dilator muscle, resulting in a smaller pupil size. Pharmacologically induced mydriasis is achieved either by stimulating the iris dilator muscle with the use of direct or indirect alpha-1 agonists (eg, phenylephrine, hydroxyamphetamine), by blocking the iris sphincter muscle with the use of muscarinic antagonists or cycloplegic drugs (eg, tropicamide) or with a combination (eg, Paremyd®, which is 1% hydroxyamphetamine hydrobromide and 0.25% tropicamide). Nyxol, either by directly antagonizing the alpha-1 receptor or by indirectly antagonizing the pupil dilation effect of muscarinic blocking, can expedite the reversal of pharmacologically induced mydriasis prior to natural reversal.
Study Objectives	The objectives of this study are: • To evaluate the efficacy of Nyxol to expedite the reversal of pharmacologically induced mydriasis across multiple mydriatic agents, with an emphasis on phenylephrine • To evaluate the efficacy of Nyxol to return subjects to baseline accommodation after worsening with cycloplegic agents tropicamide and Paremyd • To evaluate the systemic exposure of Nyxol based on pharmacokinetic (PK) sampling • To evaluate the safety of Nyxol • To evaluate any additional benefits of the reversal of pharmacologically induced mydriasis The Sponsor intends to use this Phase 3 registration study to evaluate Nyxol for the indication "the treatment of pharmacologically induced mydriasis produced by adrenergic (phenylephrine) or parasympatholytic (tropicamide) agents, or a combination thereof."

Study Design	This is a randomized, parallel-arm, double-masked, placebo-controlled Phase 3 study in approximately 330 randomized subjects evaluating the safety and efficacy of Nyxol in subjects with pharmacologically induced mydriasis. Following the successful completion of screening, each subject will be randomized to mydriatic agent (unmasked) and treatment (masked). Treatment randomization will be [redacted] (Nyxol or placebo [vehicle], respectively). Stratification by iris color will be [redacted] (light or dark irides) as shown in Appendix 1. The mydriatic agent randomization will be 3:1:1 (2.5% phenylephrine, 1% tropicamide, or Paremyd, respectively). Approximately 60% of the randomized subjects will receive 1 drop of 2.5% phenylephrine in both eyes (OU) 1 hour before treatment (198 subjects), approximately 20% will receive 1 drop of 1% tropicamide OU 1 hour before treatment (66 subjects), and approximately 20% will receive 1 drop of Paremyd OU 1 hour before treatment (66 subjects). Subjects will have 2 drops of study treatment (Nyxol or placebo) administered in the study eye [redacted]). Each drop will be instilled 5 minutes apart. Subjects will have only 1 drop of study treatment administered in the fellow eye [redacted]. The study eye and fellow eye will both be evaluated at all assessments unless otherwise specified. Blood sampling for Nyxol PK measurements will be conducted in a subset of approximately [redacted] adult subjects at approximately [redacted] select study sites.
Patient Population	Approximately 330 healthy subjects
Inclusion Criteria	1. Males or females ≥ 12 years of age 2. Ability to comply with all protocol-mandated procedures independently and to attend all scheduled office visits 3. Adults (≥ 18 years of age) willing to give written informed consent to participate in this study. Children aged 12-17 years to provide signed assent form, as well as a separate parental/Legal Guardian consent
Exclusion Criteria	**Ophthalmic (in either eye):** 1. Clinically significant ocular disease as deemed by the Investigator (eg, glaucoma, corneal edema, uveitis, severe keratoconjunctivitis sicca) that might interfere with the study 2. Unwilling or unable to discontinue use of contact lenses at screening until study completion 3. Unwilling or unable to suspend use of topical medication at screening until study completion 4. Ocular trauma, ocular surgery, or non-refractive laser treatment within the 6 months prior to screening 5. Use of any topical prescription or over-the-counter (OTC) ophthalmic medications of any kind within 7 days of screening,

	with the exception of lid scrubs with OTC products (eg, OCuSOFT® lid scrub, SteriLid®, baby shampoo, etc.) 6. Recent or current evidence of ocular infection or inflammation in either eye (such as current evidence of clinically significant blepharitis, conjunctivitis, or keratitis). Subjects must be symptom free for at least 7 days prior to screening 7. History of herpes simplex or herpes zoster keratitis 8. Closed or very narrow angle that in the Investigator's opinion is potentially occludable if the subject's pupil is dilated 9. History of any traumatic (surgical or nonsurgical) or nontraumatic condition affecting the pupil or iris 10. History of cauterization of the punctum or punctal plug (silicone or collagen) insertion or removal 11. Known allergy, hypersensitivity, or contraindication to any component of the phentolamine ophthalmic solution or to any component of the mydriatic agents or vehicle formulation 12. Prior participation in a study involving the use of Nyxol for the reversal of mydriasis **Systemic:** 13. Known hypersensitivity or contraindication to α- and/or β-adrenoceptor antagonists 14. Clinically significant systemic disease (eg, uncontrolled diabetes, myasthenia gravis, cancer, hepatic, renal, endocrine, or cardiovascular disorders) that might interfere with the study 15. 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 (Appendix 5) 16. Participation in any investigational study within 30 days prior to screening 17. 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 premenstrual, 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 and must intend to not become pregnant during the study 18. Resting heart rate (HR) outside the normal range (50-110 beats per minute) at the Screening Visit. Heart rate may be repeated only

	once if outside the normal range following at least a 5-minute rest period in the sitting position 19. Hypertension with resting diastolic blood pressure (BP) > 105 mmHg or systolic BP > 160 mmHg at the Screening Visit. Blood pressure may be repeated only once if outside the specified range following at least a 5-minute rest period in the sitting position
Visit Schedule	[redacted] Table 1 [redacted] Table 1
Number of Investigational Sites	Approximately 16 sites
Sample Size Justification	A sample size of approximately 330 subjects (approximately 220 treated with Nyxol) in this study will result in a total of > 300 subjects treated with Nyxol in the reversal of mydriasis program (including prior studies); this number of subjects is needed to meet the minimum number of subjects exposed to Nyxol to assess safety in this population. [redacted]
Primary Efficacy Endpoint	Percentage of subjects' study eyes returning to ≤ 0.2 mm from baseline (-1 hour) photopic **pupil diameter** at 90 minutes
Secondary Efficacy Endpoints	Secondary efficacy endpoints (for the study eye and the fellow eye, unless otherwise specified; and binocular for selected visual acuity measurements) will include: • Percentage of subjects returning to ≤ 0.2 mm from baseline (-1 hour) photopic **pupil diameter** at each remaining time point (0 minutes, 30 minutes, 60 minutes, 90 minutes [fellow eye], 2 hours, 3 hours, 4 hours, 6 hours, and 24 hours)

	• Change (in mm) in photopic **pupil diameter** from max pupil dilation (0 minutes) at each time point (30 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours, and 24 hours) • Time (hours) to return to ≤ 0.2 mm from baseline (-1 hour) photopic pupil diameter (**time-savings analysis**) • Percentage of subjects with unchanged **accommodation** from baseline (-1 hour) at 0 minutes, 90 minutes, 2 hours, 3 hours, and 6 hours • Change (in diopters) in **accommodation** from max pupil dilation (0 minutes) at 90 minutes, 2 hours, 3 hours, and 6 hours • Change (in mm) in the **pupillary light reflex** from max pupil dilation (0 minutes) at 90 minutes, 3 hours, and 6 hours (study eye) • Change (in letters) in best-corrected distance visual acuity **(BCDVA) under normal photopic lighting versus glare conditions** (using Brightness Acuity Tester [BAT]) from baseline (-1 hour) at 0 minutes, 60 minutes, 2 hours, and 6 hours (study eye) • Percentage of subjects with unchanged (within ± 4 letters) **BCDVA under glare conditions (BAT)** from baseline (-1 hour) at 0 minutes, 60 minutes, 2 hours, and 6 hours (study eye) • Change in **glare discomfort** from max pupil dilation (0 minutes) at 60 minutes, 2 hours, and 6 hours (study eye) Measurements: • Pupil diameter and pupillary light reflex will be measured with a NeurOptics VIP-300 pupillometer (mm) • Accommodation will be measured by the [redacted] • Glare testing will be performed using the Marco BAT 2000 [redacted] The Modified Intent-to-Treat (mITT) Population will be used for the primary endpoint analysis and to analyze selected secondary efficacy endpoints. The Per Protocol (PP) Population will be used to analyze selected secondary efficacy endpoints. Some of the efficacy endpoints will be analyzed overall, by mydriatic agent, and by light/dark irides at all time points.
Safety Endpoints	The primary safety measures are **conjunctival hyperemia, impairment in visual acuity (BCDVA and distance-corrected near visual acuity [DCNVA]), subjective ocular tolerability, and adverse events (AEs).** Other safety measures include intraocular pressure (IOP), subject questionnaire, and systemic safety as measured by HR and BP.

	Safety analyses will include: • Change from baseline (-1 hour) in the **conjunctival hyperemia** grading (Cornea and Contact Lens Research Unit [CCLRU] images) at each time point (0 minutes, 30 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours, and 24 hours) for the study eye and fellow eye • Change from baseline (-1 hour) in **BCDVA** at 0 minutes, 60 minutes, 2 hours, 6 hours, and 24 hours for the study eye, fellow eye, and binocular • Change from baseline (-1 hour) in **DCNVA** at 0 minutes, 90 minutes, 3 hours, 6 hours, and 24 hours for the study eye, fellow eye, and binocular • **Subjective ocular tolerability** at 0 minutes after administration of study medication for the study eye and fellow eye • Change from screening in **IOP** at 6 hours for the study eye and fellow eye • Change from baseline (-1 hour) in **subject questionnaire** responses at 0 minutes, 60 minutes, 2 hours, 4 hours, and 24 hours • Change from screening in **vital signs (HR and BP)** at 6 hours and 24 hours Measurements: • Conjunctival hyperemia will be assessed visually with a 4-point grading scale (0-3) using CCLRU images (Appendix 2) • BCDVA will be measured in photopic conditions by a standard Early Treatment Diabetic Retinopathy Study (ETDRS) illuminated chart (on wall or stand) at [redacted] (letters recorded) • DCNVA will be measured in photopic conditions by Near Visual Acuity Chart [redacted] at [redacted] (letters recorded) • Subjective ocular tolerability will be measured on a 4-point scale (0-3) • IOP will be measured with the Tono-Pen or Goldmann applanation tonometer • Subject questionnaire will be a brief symptom survey (Appendix 3)
Study Medications, Dose and Mode of Administration	Nyxol® Eye Drops (Phentolamine Ophthalmic Solution): 2 drops (dosed [redacted] apart) of Nyxol in the study eye (OD) (1 hour post mydriatic drug instillation) and 1 drop of Nyxol in the fellow eye (OS). Placebo (Nyxol vehicle): 2 drops (dosed [redacted] apart) of placebo in the study eye (OD) (1 hour post mydriatic drug instillation) and 1 drop of placebo in the fellow eye (OS).

Pharmacokinetics	At approximately 2 study sites, blood will be drawn from approximately 30 adult subjects) for Nyxol PK measurement on Visit 1/Day 1 at 15 minutes, 60 minutes, and 3 hours following administration of study medication. For adult subjects participating in PK sampling, a total of approximately 18 mL blood will be drawn (3 × 6 mL).
Duration of Subject Participation and Study	The total length of subject participation is [redacted] The execution of the entire study (first subject screen through last randomized subject) is expected to be approximately 4 to 6 months.

TABLE OF CONTENTS

2. INTRODUCTION

2.1. Investigational products

The test product is Nyxol® Eye Drops – 0.75% Phentolamine Ophthalmic Solution (0.75% POS) (herein referred to as Nyxol), a non-selective alpha-1 and alpha-2 adrenergic antagonist. Note that the concentration of 0.75% refers to phentolamine free base and is the same as and used in place of "1% phentolamine mesylate ophthalmic solution", which was how Nyxol had been described in all studies prior to the End of Phase 2 meeting in mid-2020. Placebo control is Nyxol vehicle alone.

2.2. Findings from nonclinical and clinical studies

Detailed findings from nonclinical and clinical studies and potential risk are provided in the Investigators' Brochure (IB) (2021).

Nyxol has been assessed in 8 investigator-initiated and sponsored Phase 1, 2, and 3 clinical trials. Across all trials, 335 of 567 subjects were exposed to at least 1 dose of Nyxol.

In prior clinical trials, Nyxol has demonstrated a consistent ability to decrease pupil diameter (PD) by approximately 20% (~1 to 2 mm) in both mesopic and photopic conditions with rapid onset and durable effects to 24 to 36 hours (OP-NYX-01a2 CSR; OP-NYX-SNV CSR; OPI-NYXG-201 CSR; OPI-NYXRM-201 CSR; Karpecki 2021; Pepose MIRA-2 2021; Pepose VEGA-1 2021; Pepose ORION 2021).

Nyxol was observed to be well tolerated in all studies at single-doses and multiple daily doses for up to 14 days. Safety of the patients in these trials was evaluated by adverse event (AE) monitoring, ophthalmic examinations, and vital sign assessments. Across all trials, there were no treatment-related serious adverse events (SAEs). 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 severity, with the most common being transient conjunctival hyperemia and ocular irritation; however, Nyxol dosing at or near bedtime was observed to mitigate or minimize these side effects during the daytime.

Phase 2 Reversal of Pharmacologically Induced Mydriasis Study (MIRA-1)

In OPI-NYXRM-201 (MIRA-1), 32 healthy subjects were randomized at Visit 1 in a 1:1 ratio to receive 1 drop of 1% Nyxol (1% Phentolamine Mesylate Ophthalmic Solution) or 1 drop of placebo (vehicle) in both eyes (OU) at Visit 1 and 1 drop of the alternative study medication OU at Visit 2. One hour before administration of study medication, subjects received 1 drop OU of a mydriatic agent (2.5% phenylephrine or 1% tropicamide) to dilate their pupils. Each subject received the same mydriatic agent throughout the study. Pupil diameter (PD) was measured 30 minutes and 1 hour, 2 hours, 4 hours, and 6 hours after dosing at Visit 1 and Visit 2 and compared to baseline.

In this study, Nyxol demonstrated a statistically significant reduction in PD from max pupil dilation (0 minutes [60 minutes after mydriatic agent]) compared to placebo at 1 hour, 2 hours, 4 hours, and 6 hours after treatment. These statistically significant (p<0.0001) and clinical meaningful (typically greater than 1 mm reduction) PD reductions were observed at each time point, including when stratified by both parasympathetic (tropicamide) and adrenergic (phenylephrine) agents.

Additionally, Nyxol was effective at returning a statistically significant percent of subjects' eyes to baseline PD compared with placebo treatment at every time point with estimable results, regardless of whether the PD threshold used was ≤ 0.5 mm from baseline (2-hour time point) or the more stringent, ≤ 0 mm from baseline (4-hour and 6-hour time points). The reduction in time needed to achieve reversal of mydriasis in either eye was statistically significant with Nyxol treatment compared with placebo treatment, regardless of the threshold examined or the mydriatic agent used. In this study, Nyxol led to an average time-savings of 2.2 hours to return PD to ≤ 0 mm from baseline compared to placebo. Additionally, 40% of subjects given phenylephrine and 30% of subjects given tropicamide achieved a time-savings of more than 4 hours with Nyxol, based on a *post-hoc* analysis.

In a *post-hoc* analysis, a statistically significant difference was observed in the percentage of subjects returning to baseline in the Nyxol group compared to the placebo group (after dilation with either phenylephrine or tropicamide) at 2 hours (29% vs 13%, respectively; p=0.03). This difference favoring Nyxol widened (68% for Nyxol and 23% for placebo) at 4 hours (p<0.0001).

In this study, there were no severe AEs, with only mild-to-moderate conjunctival hyperemia that resolved in most subjects within 6 hours.

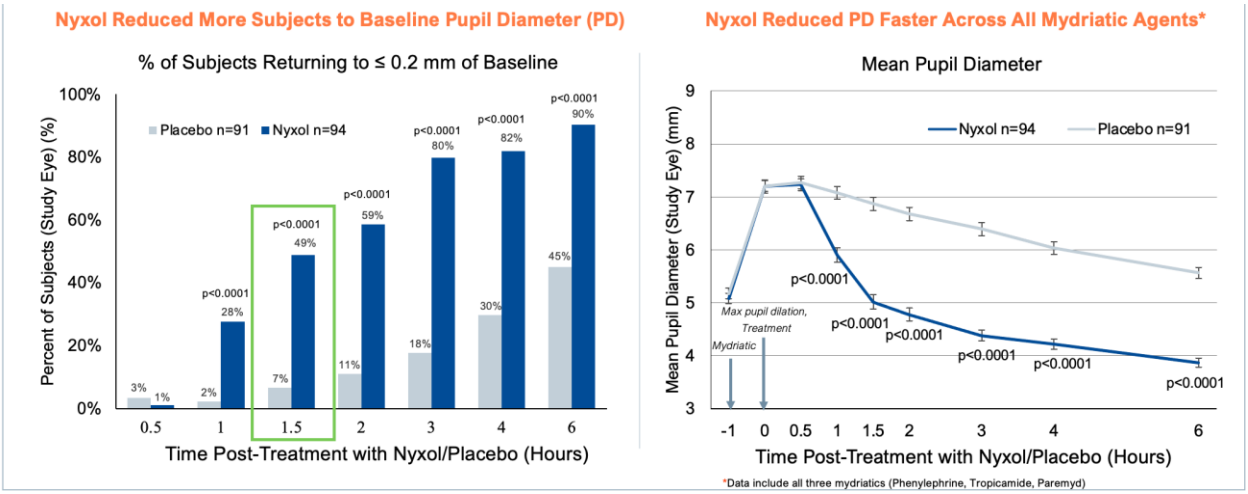
Phase 3 Reversal of Pharmacologically Induced Mydriasis Study (MIRA-2)

The OPI-NYXRM-301 study (MIRA-2) was a phase 3, multicenter, randomized, placebo-controlled, double-masked clinical trial. In MIRA-2, 185 healthy subjects aged ≥ 12 years from 12 sites were randomized 1:1 to receive Nyxol or placebo (2 drops in the study eye and 1 drop in the fellow eye), 1 hour after pupil dilation with 1 of 3 mydriatic agents (2.5% phenylephrine, 1% tropicamide, or Paremyd). The primary endpoint of an increase in the percent of study eyes returning to baseline PD at 90 minutes after Nyxol compared to placebo was met (Figure 1A [green box]). For subjects in the Modified Intent-to-Treat (mITT) Population treated with Nyxol, 49% had PD return to ≤ 0.2 mm from baseline at 90 minutes compared to only 7% of subjects treated with placebo (p<0.0001). At 60 minutes, 28% of study eyes in subjects who received Nyxol had returned to baseline PD, compared to only 2% of participants who received placebo. The differences in percent of patients returning to baseline were statistically significant across all time points from 60 minutes through 6 hours (p<0.0001; Figure 1A). Additionally, multiple secondary endpoints met statistical significance, including mean PD, which was significantly smaller with Nyxol compared with placebo at all timepoints after 30 minutes (p<0.0001; Figure **1**B). Finally, the mean time to return to baseline PD for subjects in the Per Protocol (PP) Population was ~2 hours in Nyxol-treated study eyes compared to ~6 hours in placebo-treated study eyes. Efficacy was demonstrated across the mydriatic agents and for subjects with either light or dark irides.

Nyxol treatment was well tolerated in this study. Adverse effects were limited to predominantly mild, transient hyperemia and predominantly mild discomfort on drug instillation. Reduction in PD with Nyxol did not result in a decrease in best-corrected distance visual acuity (BCDVA). There were no discontinuations due to AEs or SAEs. Vital signs were not affected by Nyxol treatment. Nyxol was also well tolerated in the pediatric population, with AEs in a single subject, who reported transient mild pain upon Nyxol instillation OU.

Figure 1: MIRA-2 Pupil Diameter Results. A. Percent of Subjects Returning to ≤ 0.2 mm From Baseline. B. Mean Pupil Diameter Across Time Points.

A. **B.**



2.3. Design justification

Pupil size is under the control of 2 opposing sets of muscles – the circular constrictor muscles controlled by the cholinergic nervous system and the radial dilator muscles, controlled by the adrenergic nervous system (Steinhauer 2004; Yoshitomi 1985). The radial dilator muscles contain predominantly α-1 adrenergic receptors that can be inhibited by α-1 antagonists (Yu 2002); therefore, it is possible to inhibit dilation of the pupil through blockade of the radial dilator muscles.

Pharmacologically induced mydriasis is achieved either by stimulating the iris dilator muscle with the use of direct or indirect alpha-1 agonists (eg, phenylephrine, hydroxyamphetamine) or by blocking the iris sphincter muscle with the use of muscarinic antagonists or cycloplegic drugs (eg, tropicamide) or with a combination (eg, Paremyd®, which is 1% hydroxyamphetamine hydrobromide and 0.25% tropicamide). Pharmacologically induced mydriasis is commonly used to facilitate clinical examination of the retina and other intraocular structures. Typically, pharmacologically induced mydriasis dilates the pupil to 6 to 8 mm, with effects lasting 6 to 24 hours. However, factors such as an individual's iris pigmentation and age cause variations in effect duration, which ranges from hours to days. During this time, individuals may experience sensitivity to light, blurred vision, or cycloplegia (loss of accommodation via the temporary paralysis of the muscle that allows the eye to focus on near objects). Accelerating mydriatic reversal after an eye exam may reduce the duration of these side effects and be beneficial for many patients.

Phentolamine is a non-selective alpha-1 and alpha-2 adrenergic antagonist acting on adrenergic receptors and is known to inhibit contraction of the iris dilator muscle, resulting in a smaller pupil size. To counteract the dilatory effects of mydriatic agent, Nyxol is proposed to be instilled in the eyes post eye exam, allowing a rapid reversal of mydriasis, thereby minimizing the duration of side effects and discomfort post exam.

Although accommodation will be studied across various mydriatic agents, a decrease in the amplitude of accommodation with mydriasis would only be expected with the use of mydriatic agents that inhibit muscarinic receptors, including tropicamide and Paremyd. These drugs elicit

cycloplegia, or paralysis of the ciliary muscle of the eye, resulting in a loss of accommodation. Phenylephrine is a sympathetic agonist that shows mydriatic effect through direct action on sympathetic nerve receptors located on the pupillary dilator muscle of the iris, and as such would not be expected to cause material changes in accommodation.

In previous reversal of pharmacologically induced mydriasis clinical studies (MIRA-1 and MIRA-2), Nyxol was effective at inducing reversal of mydriasis with both adrenergic (phenylephrine) and parasympathetic (tropicamide) mydriatic agents, as described previously. The placebo outcomes in these studies demonstrated that the natural reversal of mydriasis takes longer with tropicamide than with phenylephrine. Despite this difference, Nyxol was able to reverse mydriasis faster in the vast majority of eyes regardless of the mydriatic agent used, but it worked faster for phenylephrine (an alpha-1 agonist), as expected given the pharmacology of Nyxol (an alpha-1 and alpha-2 antagonist).

Alpha-1 adrenergic antagonists have been shown to be safe and effective for the pharmacological reversal of mydriasis. In 1990, the United States (US) Food and Drug Administration (FDA) approved Dapiprazole Hydrochloride Ophthalmic Solution 0.5% (Rev-Eyes) for this indication; however, the product was withdrawn and discontinued by the manufacturer for reasons not related to safety or efficacy. Many people who undergo pupil dilation for an annual ophthalmic examination or other ophthalmic procedure continue to request an option for rapid reversal of the mydriasis. If Nyxol is a safe and effective treatment for the reversal of mydriasis, this may provide a new option for patients who experience side effects of pupil dilation.

2.4. Route of administration, dosage regimen, and treatment period

As the intended route of administration for Nyxol is topical ocular, this is the route to be used in this study.

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

Note that 0.75% Phentolamine Ophthalmic Solution, which expresses the phentolamine mesylate concentration in free base, is the new nomenclature being used in place of “1% Phentolamine Mesylate Ophthalmic Solution”, which was how Nyxol had been described in MIRA-1.

2.5. Compliance

This study will be conducted in compliance with the protocol and in accordance with International Council for Harmonisation (ICH) Guidelines for Good Clinical Practice (GCP), the ethical principles set forth in the Declaration of Helsinki, and with the US Title 21 Code of Federal Regulations.

2.6. Study population

A sample size of approximately 330 healthy subjects ≥ 12 years of age will be randomized in a 2:1 ratio to 1 of 2 masked treatment arms (Nyxol or placebo, respectively). Randomization will be stratified 1:1 by light/dark color irides (Appendix 1) and further randomized 3:1:1 to unmasked mydriatic agent (2.5% phenylephrine, 1% tropicamide, or Paremyd, respectively). Subjects will be recruited from approximately 16 investigational sites.

3. OBJECTIVES AND PURPOSE

The MIRA-3 study is a randomized, parallel-arm, double-masked, placebo-controlled study of the safety and efficacy of Nyxol (0.75% Phentolamine Ophthalmic Solution) to reverse pharmacologically induced mydriasis in healthy subjects.

The objectives of this study are:

- To evaluate the efficacy of Nyxol to expedite the reversal of pharmacologically induced mydriasis across multiple mydriatic agents, with an emphasis on phenylephrine
- To evaluate the efficacy of Nyxol to return subjects to baseline accommodation after worsening with cycloplegic agents tropicamide and Paremyd
- To evaluate the systemic exposure of Nyxol based on pharmacokinetic (PK) sampling
- To evaluate the safety of Nyxol
- To evaluate any additional benefits of the reversal of pharmacologically induced mydriasis

The Sponsor intends to use this Phase 3 registration study to evaluate Nyxol for the indication "the treatment of pharmacologically induced mydriasis produced by adrenergic (phenylephrine) or parasympatholytic (tropicamide) agents, or a combination thereof."

4. STUDY DESIGN

This is a randomized, parallel-arm, double-masked, placebo-controlled Phase 3 study in approximately 330 randomized subjects evaluating the safety and efficacy of Nyxol in subjects with pharmacologically induced mydriasis. Following the successful completion of screening, each subject will be randomized to mydriatic agent (unmasked) and treatment (masked). Treatment randomization will be 2:1 (Nyxol or placebo [vehicle], respectively). Stratification by iris color will be 1:1 (light or dark irides) as shown in Appendix 1.

The mydriatic agent randomization will be 3:1:1 (2.5% phenylephrine, 1% tropicamide, or Paremyd, respectively). That is, approximately 60% of the randomized subjects will receive 1 drop of 2.5% phenylephrine OU 1 hour before treatment (198 subjects), approximately 20% will receive 1 drop of 1% tropicamide OU 1 hour before treatment (66 subjects), and approximately 20% will receive 1 drop of Paremyd OU 1 hour before treatment (66 subjects). Study treatment (Nyxol or placebo) will be administered OU 1 hour after mydriatic drug instillation. Subjects will have 2 drops of study treatment (Nyxol or placebo) administered in the study eye (right eye [OD]). Each drop will be instilled 5 minutes apart. Subjects will have only 1 drop of study treatment administered in the fellow eye (left eye [OS]).

The study eye and fellow eye will both be evaluated at all assessments unless otherwise specified.

Blood sampling for Nyxol PK measurements will be conducted in a subset of approximately [redacted] adult subjects at approximately 2 select study sites.

4.1. Primary and secondary endpoints

Efficacy:

The primary efficacy endpoint is the percentage of subjects' study eyes returning to ≤ 0.2 mm from baseline (-1 hour) photopic **pupil diameter** at 90 minutes.

The study eye is defined as the right eye (OD). The fellow eye (also referred to as the non-study eye in MIRA-2) is defined as the left eye (OS). Unless specified, the study eye and fellow eye will both be evaluated at all assessments. In addition, binocular will be evaluated for selected visual acuity measurements.

Secondary efficacy endpoints will include:

- Percentage of subjects returning to ≤ 0.2 mm from baseline (-1 hour) photopic **pupil diameter** at each remaining time point (0 minutes, 30 minutes, 60 minutes, 90 minutes [fellow eye], 2 hours, 3 hours, 4 hours, 6 hours, and 24 hours)
- Change (in mm) in photopic **pupil diameter** from max pupil dilation (0 minutes) at each time point (30 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours, and 24 hours)
- Time (hours) to return to ≤ 0.2 mm from baseline (-1 hour) photopic pupil diameter (**time-savings analysis**)
- Percentage of subjects with unchanged **accommodation** from baseline (-1 hour) at 0 minutes, 90 minutes, 2 hours, 3 hours, and 6 hours
- Change (in diopters) in **accommodation** from max pupil dilation (0 minutes) at 90 minutes, 2 hours, 3 hours, and 6 hours
- Change (in mm) in the **pupillary light reflex** from max pupil dilation (0 minutes) at 90 minutes, 3 hours, and 6 hours (study eye)
- Change (in letters) in **BCDVA under normal photopic lighting versus glare conditions** (using Brightness Acuity Tester [BAT]) from baseline (-1 hour) at 0 minutes, 60 minutes, 2 hours, and 6 hours (study eye)
- Percentage of subjects with unchanged (within ± 4 letters) **BCDVA under glare conditions (BAT)** from baseline (-1 hour) at 0 minutes, 60 minutes, 2 hours, and 6 hours (study eye)
- Change in **glare discomfort** from max pupil dilation (0 minutes) at 60 minutes, 2 hours, and 6 hours (study eye)

Measurements:

- Pupil diameter and pupillary light reflex will be measured with a NeurOptics VIP-300 pupillometer (mm)
- Accommodation will be measured by the Royal Air Force (RAF) Near Point Rule (measured in cm and then converted to diopters). Unchanged accommodation from baseline (-1 hour) is defined as a change from baseline value ≥ -1, as measured in diopters
- Glare testing will be performed using the Marco BAT 2000
- Glare discomfort will be measured on a 4-point scale from 0 (none) to 3 (severe)

Some of the efficacy endpoints will be analyzed overall, by mydriatic agent, and by light/dark irides at all time points. Each mydriatic agent will be analyzed individually, and an additional analysis combining 1% tropicamide and Paremyd subjects into a “tropicamide or Paremyd” group will be performed. Additional analyses may be performed to compare efficacy endpoints

between the study eye and fellow eye within the same subject as well as the age given the pediatric population.

Safety:

The primary safety measures are **conjunctival hyperemia, impairment in visual acuity (BCDVA and distance-corrected near visual acuity [DCNVA]), subjective ocular tolerability, and AEs.** Other safety measures include intraocular pressure (IOP), subject questionnaire, and systemic safety as measured by HR and BP.

Safety analyses will include:

- Change from baseline (-1 hour) in the **conjunctival hyperemia** grading (Cornea and Contact Lens Research Unit [CCLRU] images) at each time point (0 minutes, 30 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours, and 24 hours) for the study eye and fellow eye
- Change from baseline (-1 hour) in **BCDVA** at 0 minutes, 60 minutes, 2 hours, 6 hours, and 24 hours for the study eye, fellow eye, and binocular
- Change from baseline (-1 hour) in **DCNVA** at 0 minutes, 90 minutes, 3 hours, 6 hours, and 24 hours for the study eye, fellow eye, and binocular
- **Subjective ocular tolerability** at 0 minutes after administration of study medication for the study eye and fellow eye
- Change from screening in **IOP** at 6 hours for the study eye and fellow eye
- Change from baseline (-1 hour) in **subject questionnaire** responses at 0 minutes, 60 minutes, 2 hours, 4 hours, and 24 hours
- Change from screening in **vital signs (HR and BP)** at 6 hours and 24 hours

Measurements:

- Conjunctival hyperemia will be assessed visually with a 4-point grading scale (0-3) using CCLRU images (Appendix 2)
- Best-corrected distance visual acuity will be measured in photopic conditions by a standard Early Treatment Diabetic Retinopathy Study (ETDRS) illuminated chart (on wall or stand) at [redacted] (letters recorded)
- Distance-corrected near visual acuity will be measured in photopic conditions by Near Visual Acuity Chart in the [redacted] at [redacted] (letters recorded)
- Subjective ocular tolerability will be measured on a 4-point scale (0-3)
- Intraocular pressure will be measured with the Tono-Pen or Goldmann applanation tonometer
- Subject questionnaire will be a brief symptom survey (Appendix 3)

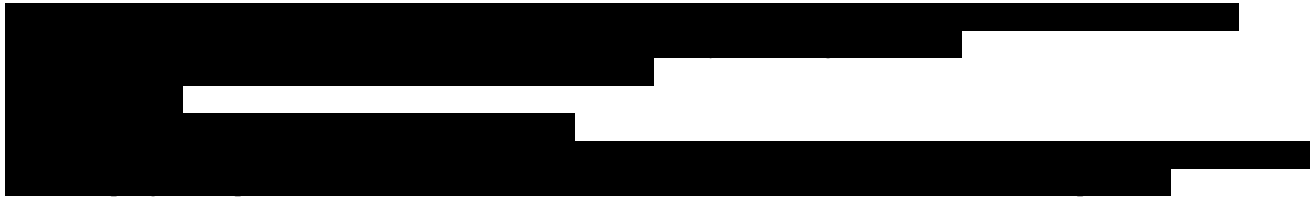
4.2. Description and schedule of visits and procedures

A sample size of approximately 330 healthy subjects ≥ 12 years of age will be randomized in a 2:1 ratio to 1 of 2 treatment arms (Nyxol or placebo, respectively). Randomization will be

stratified 1:1 by iris color (light and dark irides) and 3:1:1 by mydriatic agent (2.5% phenylephrine, 1% tropicamide, or Paremyd, respectively). Subjects will receive their mydriatic agent OU 1 hour before treatment with study medication (Nyxol or placebo). Study procedures are shown in detail in Table 1. For this study, the following time points apply:

Table 1: Screening and Mydriatic/Treatment Schedule

	[redacted]
Day	
Visit	
Start time [b]	
Informed consent/assent	
Subject identification number assigned	
Medical/Ophthalmic history	
Demographics	
Prior/Concomitant medications [c]	
Urine pregnancy test [d]	
HR/BP	
[redacted]	



4.3. Measures taken to minimize/avoid bias

This is a placebo-controlled, double-masked, 2:1 randomized (Nyxol or placebo, respectively), 2-arm, Phase 3 study. Nyxol vehicle is used as placebo.

4.4. Study medications

Study Medication Identification

Established name	Phentolamine mesylate – parent phentolamine
CAS registry number	65-28-1 – parent 50-60-2
Chemical class	An α-adrenergic 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	377.140– parent 281.352
Drug name/formulation	Nyxol / aqueous, sterile, non-preserved, isotonic, ophthalmic solution
Concentration active	1% – parent 0.75% (free acid)
Manufacturer drug substance	[redacted]
Manufacturer drug product, placebo	Woodstock Sterile Solutions, Inc.
Storage requirements	[redacted] Stored at the site [redacted] in a secured location (locked) with no access for unauthorized personnel per ICH GCP Guidelines.

Formulation

Nyxol (0.75% Phentolamine Ophthalmic Solution) is a clear, colorless to slightly brown, sterile, non-preserved, isotonic, buffered aqueous solution containing 1% phentolamine mesylate (equivalent to 0.75% phentolamine free base), mannitol, and sodium acetate. Placebo for Nyxol is a clear, colorless, sterile, non-preserved, isotonic, buffered aqueous solution containing mannitol and sodium acetate. The pH of the study medications may be adjusted with [redacted] (United States Pharmacopeia [USP]) and/or [redacted] (USP) to [redacted].

4.4.1. Packaging and labeling

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

4.4.2. Storage of study medication and dispensing

Prior to dispensing, all investigational material must be stored in a secure locked location with limited access documented by signature of authorized persons who may dispense investigational materials. [redacted] Study medication must not be frozen and must be protected from light.

4.4.3. Study medication administration

Study medication will be administered by the Investigator or designee at the site on Treatment Visit 1. To administer the study medication, the foil pouch is opened and the strip of 5 vials removed from the pouch. A single vial is removed from the strip by twisting the vial, which exposes the dropper tip and contents of the vial. The vial should be removed immediately prior to dosing to protect the contents of the vial. After administration, all used (1 vial) and unused vials (4 vials [5 vials total]) should be returned to the labeled foil pouch and the original kit box and retained at site, for proper accountability and reconciliation.

4.4.4. Study medication accountability

4.4.4.1. Receipt and disposition of study medication

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

4.4.4.2. Return of study medication

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

4.5. Expected duration of subject participation

The total length of subject participation is [redacted], with [redacted] clinic visits, as summarized below:

[redacted]

The execution of the entire study (first subject screened through last randomized subject completed) is expected to be approximately 4 to 6 months.

4.6. Randomization and procedure for breaking the code

A randomization code for allocating subjects to treatment will be prepared by an unmasked biostatistician not directly connected with the study. Randomization to study medication 2:1 (Nyxol or placebo, respectively) will be stratified 1:1 by light/dark color irides and further randomized 3:1:1 to mydriatic agent (2.5% phenylephrine, 1% tropicamide, or Paremyd, respectively) (Appendix 4). Note that the mydriatic agent administered is unmasked.

At the initiation of study-related procedures, every potential subject will be assigned a ***subject identification number***.

The study medication will be masked to both Investigator and study subjects, as well as Ocuphire. Only in case of medical emergency or occurrence of SAEs will the code be unmasked by the Medical Monitor and made available to the Investigator, Ocuphire, and/or other personnel involved in the monitoring or conduct of this study.

4.7. Collection of data

Study-specific data that have been outlined in the protocol will be entered into the clinical database by individual(s) designated by the Investigator. Data 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 found so that proper resolutions can be made and documented in the clinical database. An audit trail within the system will track all changes made to the data.

4.8. Study medication discontinuation

The study medication may be discontinued for the following reasons:

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

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

4.8.1. Reasons for withdrawal from study

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

4.8.2. Entire study terminated

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

4.8.3. Actions after discontinuation

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

For any subject who chooses to withdraw consent or who is noncompliant, every possible effort should be made by the Investigator to assure the 6-hour measurements are assessed prior to discontinuation, in addition to a follow-up telephone call that includes assessments for AEs, concomitant medications, and subject-evaluated conjunctival hyperemia.

4.9. Completed study

The study is completed when all randomized subjects have completed 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 have been resolved.

5. SUBJECT INCLUSION AND EXCLUSION CRITERIA

5.1. Subject inclusion criteria

Included in the study will be individuals with the following characteristics:

1. Males or females ≥ 12 years of age
2. Ability to comply with all protocol-mandated procedures independently and to attend all scheduled office visits
3. Adults (≥ 18 years of age) willing to give written informed consent to participate in this study. Children aged 12-17 years to provide signed assent form, as well as a separate parental/Legal Guardian consent

5.2. Subject exclusion criteria

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

Ophthalmic (in either eye):

1. Clinically significant ocular disease as deemed by the Investigator (eg, glaucoma, corneal edema, uveitis, severe keratoconjunctivitis sicca) that might interfere with the study
2. Unwilling or unable to discontinue use of contact lenses at screening until study completion
3. Unwilling or unable to suspend use of topical medication at screening until study completion
4. Ocular trauma, ocular surgery, or non-refractive laser treatment within the 6 months prior to screening
5. Use of any topical prescription or over-the-counter (OTC) ophthalmic medications of any kind within 7 days of screening, with the exception of lid scrubs with OTC products (eg, OCuSOFT® lid scrub, SteriLid®, baby shampoo, etc.)
6. Recent or current evidence of ocular infection or inflammation in either eye (such as current evidence of clinically significant blepharitis, conjunctivitis, or keratitis). Subjects must be symptom free for at least 7 days prior to screening
7. History of herpes simplex or herpes zoster keratitis
8. Closed or very narrow angle that in the Investigator's opinion is potentially occludable if the subject's pupil is dilated
9. History of any traumatic (surgical or nonsurgical) or nontraumatic condition affecting the pupil or iris
10. History of cauterization of the punctum or punctal plug (silicone or collagen) insertion or removal
11. Known allergy, hypersensitivity, or contraindication to any component of the phentolamine ophthalmic solution or to any component of the mydriatic agents or vehicle formulation
12. Prior participation in a study involving the use of Nyxol for the reversal of mydriasis

Systemic:

13. Known hypersensitivity or contraindication to α- and/or β-adrenoceptor antagonists
14. Clinically significant systemic disease (eg, uncontrolled diabetes, myasthenia gravis, cancer, hepatic, renal, endocrine, or cardiovascular disorders) that might interfere with the study
15. 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 (Appendix 5)
16. Participation in any investigational study within 30 days prior to screening
17. 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 premenstrual, 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 and must intend to not become pregnant during the study

18. Resting HR outside the normal range (50-110 beats per minute) at the Screening Visit. Heart rate may be repeated only once if outside the normal range following at least a 5-minute rest period in the sitting position
19. Hypertension with resting diastolic BP > 105 mmHg or systolic BP > 160 mmHg at the Screening Visit. Blood pressure may be repeated only once if outside the specified range following at least a 5-minute rest period in the sitting position

6. TREATMENT OF SUBJECTS

Approximately 330 healthy subjects ≥ 12 years of age will be randomized in a 2:1 ratio to 1 of 2 treatment arms (Nyxol or placebo, respectively). Randomization will be stratified 1:1 by light/dark color irides and further randomized 3:1:1 to mydriatic agent (2.5% phenylephrine, 1% tropicamide, or Paremyd, respectively). [redacted]

6.1. Treatment adherence

All subjects will be treated by the Investigator or designee at the study clinic on Visit 1.

6.2. Concomitant medications

As noted in the exclusion criteria (Section 5.2), use of any topical prescription or OTC ophthalmic medications of any kind within 7 days of screening is prohibited, with the exception of lid scrubs with OTC products (eg, OCuSOFT lid scrub, SteriLid, baby shampoo, etc.) which may have been used prior to but not at screening until study completion.

Additionally, initiation of treatment with or any changes to the current dosage, drug, or regimen of any systemic adrenergic or cholinergic drugs (Appendix 5) within 7 days prior to screening or during the study is prohibited. However, a subject can be treated with a systemic adrenoceptor antagonist, for example, as long as the particular agent and its dose and regimen had been consistent for the 7 days prior to screening, and there was no reason to believe that alteration would be necessary at some point later during the study.

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

Use of all medications should be documented on the appropriate eCRF. Investigators are encouraged to contact the Medical Monitor for any questions regarding allowed medications. Judgment of continued study participation by the subject, and inclusion of this subject's subsequent visits in the safety and efficacy analysis will be made by Ocuphire.

All medications taken by the subject 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®), the brand name is required. For non-combination products, the generic name

is desired. The use of routine ophthalmic diagnostic pharmaceutical agents (eg, fluorescein and local anesthetic) will be allowed and should be documented. Any change in dosing parameters should also be recorded in the eCRF.

7. ASSESSMENT OF EFFICACY

7.1. Specification of the efficacy parameters

The primary efficacy endpoint is the percentage of subjects' study eyes with 2 drops of treatment returning to ≤ 0.2 mm baseline (-1 hour) photopic pupil diameter at 90 minutes.

The study eye is defined as the right eye (OD). The fellow eye is defined as the left eye (OS). Unless specified, the study eye and fellow eye will both be evaluated at all assessments. Specified endpoints will also be analyzed for both eyes. Secondary efficacy endpoints can be found in Section 4.1.

The mITT Population will be used for the primary endpoint analysis and to analyze selected secondary efficacy endpoints. The PP Population will be used to analyze selected secondary efficacy endpoints. Some of the efficacy endpoints will be analyzed at all time points overall, by mydriatic agent, and by light/dark irides.

7.2. Assessing, recording, and analyzing efficacy parameters

Pupil diameter will be measured at Treatment Visit 1 and at Follow-Up Visit 2. Accommodation, pupillary light reflex, glare testing (BAT), and glare discomfort will all be measured only at Treatment Visit 1.

- Pupil diameter and pupil reactivity will be measured with the NeurOptics VIP-300 pupillometer (mm)
- Accommodation will be measured by the RAF Near Point Rule ( ). Unchanged accommodation from baseline (-1 hour) is defined as a change from baseline value ≥ -1, as measured in diopters
- Glare testing will be performed using the Marco BAT 2000
- Glare discomfort will be measured on a 4-point scale from 0 (none) to 3 (severe)

The photopic lighting conditions in the room will be defined as lights off with ambient light coming from the distance ETDRS illuminated chart (on wall or stand) 4 m away at a luminance level of 85 cd/m^2 (or 85 lux). Subjects will be allowed to acclimate to these lighting conditions (with the eyes open normally for a minimum of 2 minutes) prior to the PD, BCDVA, and DCNVA (safety measures) measurements at all scheduled time points. Subjects will sit in the exam chair facing the illuminated chart during the acclimation period and for all assessments. Scotopic PD will be measured OD with room lights and illuminated chart off and OS occluded. BAT testing will be performed OD with OS occluded. Room lights will be off with the distance ETDRS chart illuminated. Room lights should be turned on for accommodation measurements and for the remaining safety assessments (eg, conjunctival hyperemia, AEs, brief subject questionnaire, etc.). The subject will be in the same room for all assessments, and every effort will be made to have the same person perform the measurements at all time points.

Some of the efficacy endpoints will be analyzed overall, by mydriatic agent, and by light/dark irides at all time points. Each mydriatic agent will be analyzed individually, and an additional analysis combining 1% tropicamide and Paremyd subjects into a "tropicamide or Paremyd"

group will be performed. Exploratory analyses may be performed to compare efficacy endpoints between the study eye and fellow eye within the same subject.

7.2.1. Screening/Day 1

Individuals who are potential subjects are identified by the study center to schedule the Screening Visit. The Screening Visit should occur the same day as Treatment Visit 1, when the dose of study treatment is given.

Once subjects arrive at the study center, a member of the study center staff will interview the individual as to their qualifications for participation in the study, and if the subject wishes to continue, the informed consent form is signed, and a subject identification number is assigned. Children aged 12 to 17 years must provide assent. Screening includes an explanation of the study, a medical and ophthalmic history, demographics, a review of prior/concomitant medications, a urine pregnancy test (for females of childbearing potential), and HR/BP assessment. The second step in screening includes procedures such as IOP assessment and an ophthalmic examination consisting of biomicroscopy and direct or indirect ophthalmoscopy without dilation.

Investigators are cautioned to appropriately note all observations of conjunctival hyperemia (also called conjunctival erythema) on the biomicroscopy eCRF at screening.

7.2.2. Treatment Visit 1/Day 1

Treatment Visit 1 should be the same day as the Screening Visit. Once the subject has completed the screening assessments part of the visit and it is confirmed that he/she meets all of the inclusion criteria but none of the exclusion criteria, the visit will then transition to the Treatment Visit 1 assessments. The study eye is OD and the fellow eye is OS.

As part of the Treatment Visit 1, the subject:

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

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

7.2.3. Follow-Up Visit/Day 2

On Day 2, the subject will return to the clinic for a Follow-Up Visit.

As part of the Follow-Up Visit, the subject will be assessed at [redacted]

7.2.4. Unscheduled Visits

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

As noted in Section 4.8.3, every possible effort should be made by Investigators to assure that subjects who discontinue early from the study have a telephone follow-up that includes assessments of AEs, concomitant medications, and subject-evaluated conjunctival hyperemia.

7.2.5. Visit Variation

Visits on Day 2 may occur between 24 to 30 hours after the baseline (-1 hour time point on Treatment Day 1).

8. PHARMACOKINETIC ASSESSMENTS

For Nyxol PK analysis at Treatment Visit 1, blood samples will be collected to establish drug levels of Nyxol from approximately 30 adult subjects at approximately 2 select study sites. Six mL of blood will be drawn 15 minutes, 60 minutes, and 3 hours post dose. Analysis of plasma samples for Nyxol concentration determinations will be performed by a central PK laboratory using a validated liquid chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.

9. ASSESSMENT OF SAFETY

9.1. Specification of safety parameters

The assessment of safety and tolerability is a secondary objective of this study. The assessment of safety will be performed by:

- Conjunctival hyperemia measured with CCLRU images using a 4-point scale (0-3) (Appendix 2).
    - None (0) = Normal; appears white with a small number of conjunctival blood vessel easily observed
    - Mild (+1) = Prominent, pinkish-red color of both the bulbar and palpebral conjunctiva
    - Moderate (+2) = Bright, scarlet red color of the bulbar and palpebral conjunctiva
    - Severe (+3) = Beefy red with petechiae, dark red bulbar and palpebral conjunctiva with evidence of subconjunctival hemorrhage
- Subjective ocular tolerability measured on a 4-point scale
    - 0 – No discomfort
    - 1 – Mild discomfort
    - 2 – Moderate discomfort
    - 3 – Severe discomfort
- Best-corrected distance visual acuity will be measured in photopic conditions by a standard [redacted] at [redacted] (letters recorded)
- Distance-corrected near visual acuity will be measured binocularly in photopic conditions by Near Visual Acuity Chart in the [redacted] (letters recorded)
- Intraocular pressure will be measured with the Tono-Pen or Goldmann applanation tonometer
- Heart rate and BP (as per the site's normal equipment and procedures)
- Adverse events
- Brief subject questionnaire (Appendix 3)

9.2. Assessing, recording, and analyzing safety parameters

The timing for recording safety parameters may be found in Section 4.2.

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 treatment-emergent adverse events (TEAEs)/adverse reactions will be summarized (Section 10.3.6).

All AEs/adverse reactions occurring during the study (ie, once the subject has signed the informed consent/assent) **must** be documented, regardless of the assumption of causal

relationship, on the respective eCRF. All TEAEs/adverse reactions must be documented from the time the subject receives the study medication until the subject's participation in the study has been completed. If a subject has ongoing TEAEs/adverse reactions at the time of study completion or discontinuation from the study, the ongoing TEAEs/adverse reactions are to be followed 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 considered drug related. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory findings), symptom, or disease temporarily associated with the use of the study medication, whether or not related to the study medication. Study medication includes the investigational drug under evaluation and the comparator product or vehicle placebo that is given or administered during any phase of the study.

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

The occurrence of AEs should be sought by open-ended questioning of the subject at each visit during the study. At each clinic assessment/visit, study personnel should ask the following question: "Have you had any problems since your last assessment/visit?". 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 patient or 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 Application 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; or, if an IB is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. "Unexpected," as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

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

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

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

If the relationship between the AE/SAE and the study medication is determined by Ocuphire to be anything other than "Not related" or "Unlikely related" the event will be considered to be related to the study medication 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.

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 recuperated
- Recovered/resolved: One of the possible results of an AE outcome that indicates that the event has improved or 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 informed consent/assent and until the Follow-Up Visit (Day 2). Any SAEs "suspected" to be related to the study medication and discovered by the Investigator at any time **after** the study should be reported.

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

It is the Investigator's responsibility to notify the approving IRB of any SAEs on a timely basis as instructed by 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, not drug related, expected, or unexpected (based on the available information in the IB).

Any death occurring during the study and follow-up period must be reported as an SAE. For any death occurring through the end of the study, regardless of the degree of relationship to study medication, the SAE resulting in the death must be reported to the CRO. A death occurring after completion of the study including the Safety Follow-up Visits, 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

A detailed presentation of the statistical approach is outlined in the Statistical Analysis Plan.

10.1. Sample size

A sample size of approximately 330 subjects (approximately 220 treated with Nyxol) in this study will result in a total of > 300 subjects treated with Nyxol in the reversal of mydriasis program (including prior studies); this number of subjects is needed to meet the minimum number of subjects exposed to Nyxol to assess safety in this population. The primary efficacy endpoint will be met if subjects show a positive effect for [redacted]). A total of 330 subjects (Nyxol, n=220; placebo, n=110) will [redacted] The assumptions for this power calculation are estimated from the MIRA-2 Phase 3 results.

All subjects will be randomized into the study in a 2:1 ratio to 1 of the 2 treatment arms (Nyxol or placebo, respectively), with a 1:1 stratification by light/dark irides (equal number of light and dark irides stratified across treatment groups). Furthermore, subjects will be randomized into the study at a ratio of 3:1:1 to mydriatic agent (2.5% phenylephrine, 1% tropicamide, or Paremyd, respectively). Therefore, if 198 subjects are randomized to 2.5% phenylephrine, then 66 subjects will be assigned to 1% tropicamide and 66 subjects to Paremyd, resulting in 330 total subjects.

10.2. Analysis Populations

Modified Intent-to-Treat Population: The mITT Population will include all randomized subjects who received 2 drops of study treatment in the study eye and had at least 1 scheduled post-treatment PD measurement during Visit 1. The mITT Population will be used for the primary endpoint analysis and to analyze selected secondary efficacy endpoints, with subjects included in their randomized treatment regardless of the treatment they actually received.

Per Protocol Population: The PP Population includes all subjects in the mITT Population who had 2 drops of study treatment in the study eye, had all scheduled PD measurements during Visit 1, had an increase of > 0.2 mm in PD in the study eye at 0 minutes compared to baseline (-1 hour), and had no major protocol deviations. The PP Population will be used to analyze selected secondary efficacy endpoints, with subjects included in their randomized treatment regardless of the treatment they actually received.

All Randomized Population (ARP): The ARP will include all randomized subjects. This population is also known as the Intent-to-Treat (ITT) Population. The ARP will be used in confirmatory efficacy analyses, with subjects included in their randomized treatment regardless of the treatment they actually received.

Safety Population (SP): The SP will include all randomized subjects who received at least 1 drop of study treatment. The SP will be used to summarize safety variables, using the treatment they actually received.

Pharmacokinetic Population: The PK Population will include all subjects who had at least one PK sample taken at any post-treatment timepoint.

10.3. Statistical methods

10.3.1. General considerations

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

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

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

10.3.2. Demographic and baseline characteristics

Demographic and baseline characteristics such as age, race, and sex will be summarized by treatment group using the mITT Population, PP Population, SP, PK Population, and the ARP. 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) and will be summarized by treatment group using the SP.

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 WHODrug and will both be summarized by treatment group 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, imputation will be performed for missing data as described in the Statistical Analysis Plan. If the analysis using the mITT Population shows a positive effect for Nyxol at the 0.05 level of significance, the primary endpoint will be considered met. 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.

For all efficacy endpoints, baseline is defined as -1 hour prior to treatment on Visit 1. Max pupil dilation is defined as 0 minutes, during which maximum PD is expected.

All efficacy data will be summarized by treatment group, study day, and time point (-1 hour [baseline], 0 minutes, 30 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours, and 24 hours), as appropriate.

The primary efficacy endpoint is the percentage of subjects returning to ≤ 0.2 mm from baseline (-1 hour) photopic pupil diameter at 90 minutes post treatment in the study eye. Subjects who did not have an increase of > 0.2 mm in pupil diameter in the study eye at the max pupil dilation (0 minutes) will not be counted in the PP Population as returning to ≤ 0.2 mm from baseline pupil diameter. The primary efficacy endpoint will be analyzed using a logistic regression model with treatment, mydriatic agent, and light/dark irides as factors and the baseline pupil diameter as a covariate. The percentage of subjects in each treatment group meeting the criteria and 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 of the primary efficacy endpoint will be completed using a logistic regression model with treatment as a factor and the baseline pupil diameter as a covariate; the mydriatic agent and irides type are excluded from this model.

Secondary efficacy endpoints are indicated in Section 4.1.

Each of the continuous secondary efficacy endpoints will be analyzed using analysis of covariance (ANCOVA), with change from baseline as the dependent variable, treatment, mydriatic agent, and light/dark irides as factors, and the respective baseline (-1 hour) value included as the covariate. Note that most secondary efficacy endpoints are in relation to baseline (-1 hour), whereas some endpoints are in relation to max pupil dilation (0 minutes). Each ANCOVA will be performed using the mITT or PP Populations (as specified in the Statistical Analysis Plan), 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 both treatment groups, along with the placebo-corrected LSM, its 95% CI, and associated p-value.

A comparison of the study eye and fellow eye for each subject will be completed for the primary efficacy endpoint, as well as by mydriatic agent and light/dark irides.

For each of the secondary endpoints related to percentage of subjects achieving certain criteria, the analysis will be performed using a logistic regression model with treatment, mydriatic agent, light/dark irides as factors, and the respective baseline as a covariate. For each analysis, the percentage of subjects in each treatment group meeting the criteria and 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.

The analysis of the time (hours) to return to ≤ 0.2 mm from baseline pupil diameter (time-savings analysis) endpoint will be performed using a Cox proportional hazards regression model, with treatment, mydriatic agent, light/dark irides as factors, and the baseline pupil diameter as a covariate.

In addition, secondary efficacy endpoints will be analyzed by light/dark irides and by mydriatic agent using the same model indicated above but without irides or mydriatic agent as a factor, as appropriate. Each mydriatic agent will be analyzed individually, and an additional analysis

combining 1% tropicamide and Paremyd subjects into a "tropicamide or Paremyd" group will be performed. Other subgroups, such as age, sex, and race, may be analyzed as well. If there is sufficient sample, analysis of selected efficacy endpoints will be completed for the subgroup of pediatric subjects.

10.3.6. Analysis of safety

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

Safety endpoints are indicated in Section 4.1.

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 group and time point (screening, 6 hours, and 24 hours).

Observed values and change from baseline (-1 hour) in conjunctival hyperemia at each time point (0 minutes, 30 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours, and 24 hours) will be summarized for the study eye and the fellow eye. Treatments will be compared using the same ANCOVA model proposed for the continuous secondary efficacy endpoints. Conjunctival hyperemia will also be summarized categorically.

Visual acuity assessments (BCDVA and DCNVA) will also be summarized at selected time points (BCDVA at 0 minutes, 60 minutes, 2 hours, 6 hours, and 24 hours; DCNVA at 0 minutes, 90 minutes, 3 hours, 6 hours, and 24 hours) using the number of letters correctly identified. Letters will be recorded and may later be converted to logMAR by programming for such analyses. One letter is equivalent to 0.02 logMAR. As a reference five letters is represented by 1 line. Treatments will be compared using the same ANCOVA model proposed for the continuous secondary efficacy endpoints. Distance-corrected near visual acuity will also be analyzed by mydriatic agent using a model that does not include the mydriatic agent as a factor.

For IOP, baseline is defined as the screening value. Observed values and change from baseline in IOP at 6 hours will be summarized for the study eye and the fellow eye. Treatments will be compared using the same ANCOVA model proposed for the continuous secondary efficacy endpoints.

Ocular tolerability values will be summarized by treatment group at max pupil dilation (0 minutes). Additionally, the categories "No Discomfort" and "Mild Discomfort" will be pooled into a single category and summarized descriptively, as will the categories "Moderate Discomfort" and "Severe Discomfort". Treatments will be compared for the 2 pooled categories using a Fisher's exact test.

Subject questionnaire values will be summarized by treatment group (overall and by mydriatic agent) and time point (-1 hour, 0 minutes, 60 minutes, 2 hours, 4 hours, and 24 hours).

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 be 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. Safety tables and listings for pediatric subjects may be provided.

10.3.7. Analysis of Pharmacokinetics

Nyxol plasma concentrations will be summarized by nominal sampling time using descriptive statistics. Individual subject PK data will be listed.

10.4. Procedure for accounting for missing, unused, or spurious data

For the summarization and analysis of the primary efficacy endpoint, imputation will be performed for missing data as described in the Statistical Analysis Plan. For the summarization and analysis of secondary efficacy endpoints and safety data, observed case data only will be used.

10.5. Procedure for reporting deviations from the statistical plan

Any deviations from the Statistical Analysis Plan 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 on-site, written, and telephone communications between personnel at the Investigator's site and the Medical Monitor. Should the COVID-19 pandemic restrict monitors from traveling to a site, remote review will be conducted to the extent possible, while still ensuring the study is monitored appropriately per applicable regulations and guidelines. The Investigator will allow Ocuphire, the study monitor, and the Medical Monitor to inspect all eCRFs, subject records (source documents), signed consent/assent forms, records of study medication receipt, storage, preparation, and 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, 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/assent must be approved by the IRB prior to initiation of the study. Written IRB approval must adequately identify the protocol and informed consent/assent. In addition to approving the protocol, the IRB must also approve the Subject Information and Consent/Assent/Parental

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 its designee and the IRB should be contacted.

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

Changes implemented without prior approval will be considered protocol violations.

13.4. Informed consent and assent requirements

Written informed consent will be obtained from each adult subject. A signed assent form will be obtained for all minors ages 12 to 17, as well as a separate parental/Legal Guardian consent. A copy of the signed and dated consent/assent document will be given to each subject or parent guardian. The original signed and dated informed consent/assent document must be maintained in the study files at the Investigator's site.

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

It should be emphasized that the subject is at liberty to withdraw consent/assent to participate at any time, without penalty or loss of benefits to which the subject is otherwise entitled. Subjects who refuse to give or withdraw written informed consent/assent 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 and/or legal guardian will be required to sign and date the informed consent form.

A copy of the signed and dated consent/assent document will be given to each subject. The original signed and dated informed consent/assent document must be maintained in the study

files at the Investigator's site. Signed informed consent/assent must be obtained prior to the conductance of any study procedures.

14. DATA HANDLING AND RECORD KEEPING

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

14.1. Data entry

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

14.2. Data quality control and reporting

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

14.3. Archiving of data

Archived versions of the database will be saved by 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).

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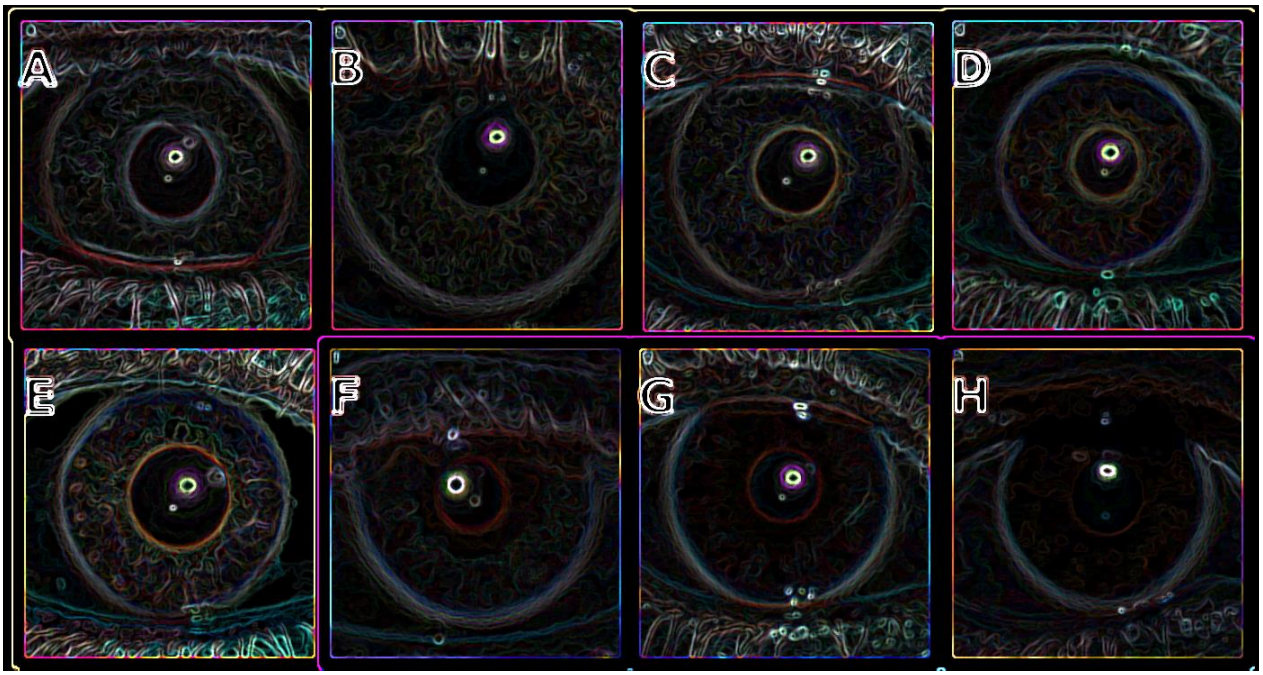
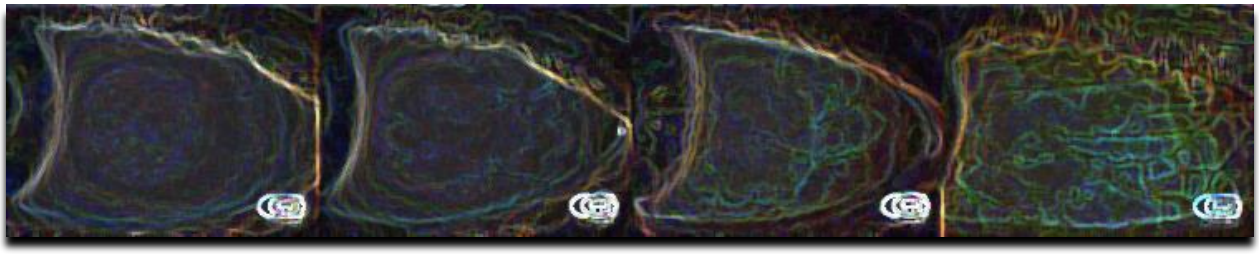
APPENDIX 1: IRIS COLOR CHART

Study enrollment includes both light- and dark-colored eyes**.** Examples of light irides ([redacted]) and dark irides ([redacted]) for the purposes of this study are detailed in the chart below.



[redacted]

APPENDIX 2: CONJUNCTIVAL HYPEREMIA GRADING SCALE USING IMAGES FROM CCLRU



APPENDIX 3: SUBJECT QUESTIONNAIRE

Study: OPI-NYXRM-302 (MIRA-3)
Subject Questionnaire

Subject Number: _____ Visit#___, Date_____
Assessment Time point _____

For this questionnaire, if you normally wear glasses to read, please put them on now.
Please read the following paragraph without squinting, and then answer the following questions.

Sunset is the time of day when our sky meets the end of the horizon.
There are blue, pink, and purple swirls spinning and twisting in the sky.
There is a coolness, a calmness, when the sun does set.

1. Circle the number below to describe the severity of your symptoms while reading the above paragraph.

[redacted]

2. For the following statement, circle the number to indicate whether you agree or disagree.

[redacted]

[redacted]

4. For the following question, circle the number to indicate how you would respond.

[redacted]

APPENDIX 4: RANDOMIZATION SCHEMA BY INVESTIGATIONAL TREATMENT, MYDRIATIC AGENT, AND IRIS COLOR

All subjects	1:1 iris color	3:1:1 mydriatic agent	2:1 treatment
330	165 Light	99 Phenylephrine	66 Nyxol
			33 Placebo
		33 Tropicamide	22 Nyxol
			11 Placebo
		33 Paremyd	22 Nyxol
			11 Placebo
	165 Dark	99 Phenylephrine	66 Nyxol
			33 Placebo
		33 Tropicamide	22 Nyxol
			11 Placebo
		33 Paremyd	22 Nyxol
			11 Placebo

APPENDIX 5: ADRENERGIC AND CHOLINERGIC DRUGS

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

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