CONFIDENTIAL

Clinical Study Phase 3 Protocol
OPI-NYXRM-301
MIRA-2

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:	**02**
Original:	**August 20, 2020**
Amendment 1:	**February 5, 2021**

SUMMARY OF CHANGES FOR AMENDMENT 1

Bold underlined text shows additions; ~~strikethrough text~~ shows deletions. For changes that affect multiple sections of the protocol, the change is listed once at the first instance below, and each subsequent protocol section incorporating that change is also listed at that point. Administrative and minor editing changes not affecting the content or conduct of the protocol have been made; these are not listed. None of the changes made affect the risks to the subjects in this study.

Section: Location	Description of Change	Rationale for Change
Synopsis: Design, p.11	A randomized, parallel arm, double-masked, placebo-controlled Phase 3 study in at least 168 randomized subjects (160 ~~completed~~ **evaluable for efficacy**), evaluating the safety and efficacy of Nyxol in subjects with pharmacologically-induced mydriasis.	Global change to clarify that evaluable subjects, which are defined within the protocol, will be used for efficacy analysis
Synopsis: Design, p.11	That is, approximately 60% of the rand mized subjects will receive one drop of 2.5% phenylephrine 1 our before treatment (96 ~~completed~~ **evaluable** subjects), approxima ely 20% will receive one drop of 1% tropicamide 1 ho r before treatment (32 ~~completed~~ **evaluable** subjects), and approximately 20% will receive Paremyd 1 hour before treatment (32 ~~completed~~ **evaluable** subjects).	
Synopsis: Patient Population, p.12	168 randomized (160 ~~completed~~ **aluable**) healthy subjects.	
Synopsis: Visit Schedule, p.14	Randomization will be stratified 3:1:1 by mydriatic agent (2.5% phenylephrine, 1% tropica ide, and Paremyd), with randomization into ea h mydriatic agent capped at approximately 96:32:32 ~~completed~~ **evaluable** subjects.	
Synopsis: Estimated Total Sample Size, p.14	Approximately 168 randomized healthy subjects, with approximately 160 ~~completed~~ **evaluable** subjects.	
Synopsis: Sample Size Justification, p.14 9.1 Sample Size, p.41	A sample si e of 160 ~~completed~~ **evaluable** subjects (80 per tr tment group) is needed for the study. The primary efficacy endpoint will be met if subjects show a positive effect for Nyxol ($\alpha = 0.05$ significance, two-tailed). One hundred and sixty ~~compl ted~~ **evaluable** subjects will provide [redacted] between the Nyxol and placebo arms in percentage of subjects returning to ≤ 0.2 mm baseline pupil diameter at 90 minutes.	
Synopsis: Duration of Subject Participation and Study, p.17	The execution of the entire study (first subject screen through last randomized subject ~~completed~~ **evaluable**) is expected to be approximately 3 to 6 months.	
4.10 Evaluable subjects, p.30	**4.10 Evaluable subjects** **Subjects are evaluable for efficacy if they received one or two drops of study treatment and had a pupil diameter measurement at the 90-minute time point at Visit 1.**	
Appendix 2, p.49	RANDOMIZATION SCHEMA **FOR EVALAUBLE SUBJECTS** BY INVESTIGATIONAL TREATMENT, MYDRIATIC AGENT, AND IRIS COLOR	

Synopsis: Secondary Efficacy Endpoints, p.15 4.1 Primary and secondary endpoints, p.24 7.2 Assessing, recording, and analyzing of efficacy parameters, p.34	Subject will be allowed to acclimate to these lighting conditions (with the eyes open normally for a minimum [redacted]) prior to the pupil diameter, BCDVA and DCNVA (safety measures)~~, and accommodation measurements~~ at all scheduled timepoints. Subject will sit in the exam chair facing the illuminated chart during the acclimation period and for all assessments [redacted] **and** the ~~scheduled~~ remaining safety assessments (e.g. [redacted], adverse events, subject questionnaire, etc.).	To clarify that room lights should be on during accommodation measurements
9.2 Analysis Populations, p.41	Per Protocol Population (PP): The per protocol (PP) population include~~ds~~ all subjects in the ~~FAS~~**mITT** [redacted], had all scheduled pupil diameter measurements during Visit 1, **had an increase of > 0.2 mm in PD in the study eye at Time 0 minutes compared to Baseline (Time –1 hour),** and had no major protocol d viations. The PP population will be used ~~for the primary endpoint ana ysis and~~ to analyze **selected secondary** efficacy endpoints.	To clarify the PP population as having an increase of > 0.2 mm in PD in the study eye at Time 0 minutes compared to Baseline
Synopsis Secondary Efficacy Endpoints, p.15 4.1 Primary and secondary endpoints, p.23	Secondary efficacy endpoints (for the study eye~~:~~ **and** for the non-study eye~~: and for the best of either eye~~) will include:	Global change per FDA feedback to define and use the mITT population as well as imputation methods for missing data for primary efficacy analysis. To harmonize the protocol with the final version of the Statistical Analysis Plan (V1.0)
Synopsis: Secondary Efficacy Endpoints, p.16 4.1.1 Primary and secondary endpoints, p.24 7.1 Specification of the efficacy parameters, p.33	~~All of the efficacy endpoints will be analyzed by Full Analysis Set (FAS) and Per Protocol (PP).~~**The Modified Intent-to-Treat (mITT) will be used f r the primary endpoint analysis and to analyze selected sec ndary efficacy endpoints. The Per Protocol (PP) population will be used to analyze selected secondary efficacy endpoints Some ~~All~~**of the efficacy endpoints will be analyzed overall, by mydriatic agent, and by light/dark irides.	
9 STATISTICS, p.41	**A detailed presentation of the statistical approach is outlined in the Statistical Analysis Plan.**	
9.2 Analysis Populations, p.41	~~Full Analysis Set~~ **Modified Intent to Treat** (~~FAS~~**mITT**): The ~~FAS~~**mITT** will include all randomized subjects who received one or two drops of study treatment and then had at least one scheduled pupil diameter measurement during Visit 1. The ~~FAS~~**mITT** will be used **for the primary endpoint analysis and** to analyze **selected secondary** efficacy endpoints.	
9.3.2 Demographics and baseline characteristics, p.42	Demographic and Baseline characteristics such as age, race and sex, will be summarized by treatment group using the ~~FAS~~**mITT**, PP, **SP,** and the ARP.	
9.3.4 Medical history and prior/concomitant medications, p.42	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 ~~FAS~~**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 ~~FAS~~**SP**.	
9.3.5 Analysis of efficacy, p.42	Efficacy will be assessed using the ~~FAS~~**mITT** and PP with subjects included in the treatment arm in which they were randomized.	

9.3.5 Analysis of efficacy, p.42	For the analysis of the primary efficacy endpoint, ~~observed case data will be used (no imputation will be performed for missing data) for the primary analysis~~ **imputation will be performed for missing data as described in the Statistical Analysis Plan**.	
9.3.5 Analysis of efficacy, p.42	~~If warranted, confirmatory analyses using the ARP with imputation for missing data will also be performed for the secondary efficacy endpoints.~~	
9.3.5 Analysis of efficacy, p.43	The analysis will be performed using the ~~FAS~~**mITT** and PP, with subjects included in their randomized treatment regardless of the treatment they actually received.	
9.3.5 Analysis of efficacy, p.43	Each ANCOVA will be performed using the ~~FAS~~**mITT** and PP with subjects included in their randomized treatment regardless of the treatment they actually received.	
9.3.5 Analysis of efficacy, p.43	For these endpoints, the ~~FAS~~**mITT** and PP will be used with subjects included in their randomized treatment regardless of the treatment they actually received.	
9.3.5 Analysis of efficacy, p.43	**A comparison of the study and non-study eye for each subject will be completed for the primary effica y endpoint, as well as by mydriatic agent.**	
9.3.5 Analysis of efficacy, p.43	Other subgroups, such as age, sex, and ra e may be analyzed as well. If there is sufficient sample, analysis of ~~safety and~~**selected** efficacy endpoints will be completed for the subgroup of pediatric subjects.	
9.3.5 Analysis of efficacy, p.44	Observed values and change from baseline (-1 hour) in conjunctival hyperemia at each timepoint (0 min, 30 minutes, 60 minutes, 90 minutes, 2 hours, 3, hours, 4 hours, 6 hours, and 24 hours), will be summarized for the study ye~~,~~ **and** the non-study eye~~, and the best of either eye~~. Treatments will be compared using the same ANCOVA model pr posed for the continuous secondary efficacy endpoints. [redacted] Visual acuity ass ssments (BCDVA and DCNVA) will also be summarized t each timepoint (0 minutes, 90 minutes, 6 hours, and 24 h urs) using letters~~,~~ **and** logMAR units~~, and the number of lines read~~. Letters will be recorded, and later converted to logMAR~~ and number of lines read~~ by programming for such analyses. One letter is equi alent 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. [redacted]. 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 non-study eye~~, and the best of either eye~~.	
9.4 Procedure for accounting for missing, unused, or spurious data, p.44	For the summarization and analysis of **the primary** efficacy ~~data~~ **endpoint**, ~~the focus will be on observed case data only. No imputation will be performed for missing data. As appropriate, confirmatory efficacy analyses will be performed using imputation for missing data; details of the imputation, if performed, will be included in the study Statistical Analysis Plan~~ **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.	

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
Study Number:	OPI-NYXRM-301
Original Protocol:	August 20, 2020
Amendment 1:	February 5, 2021

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

[redacted]

[redacted]
Tel:
Tel:
Email:

[redacted]

Signature

February 5, 2021
Date

[redacted]

Tel.
Email:

[redacted]

Signature

05Feb2021
Date

INVESTIGATOR'S AGREEMENT

OPI-NYXRM-301
MIRA-2

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: **02**

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Investigator Agreement:

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

Signature: _____ Date: _____

Printed Name: _____

PROCEDURES IN CASE OF EMERGENCY

EMERGENCY CONTACT INFORMATION

Role in Study	Name	Contact Information
Clinical Study Leader	[redacted]	[redacted] Email: [redacted]
Medical Monitor	[redacted]	[redacted] Email: [redacted]

ABBREVIATIONS AND TERMS

Abbreviation	*Full term*
AE	Adverse Event
ANCOVA	Analysis of Covariance
ARP	All Randomized Population
BCDVA	Best Corrected Distance Visual Acuity
BP	Blood Pressure
°C	Degree Centigrade
CCLRU	Cornea and Contact Lens Research Unit
CFR	Code of Federal Regulations
CHF	Congestive Heart Failure
CI	Confidence Interval
CRO	Clinical Research Organization
CSR	Clinical Study Report
°F	Degree Fahrenheit
DB	Database
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
IND	Investigational New Drug Application
IOP	Intraocular Pressure
IRB	Institutional Review Board
ITT	Intent-To-Treat

IUD	Intra-Uterine Device
LDPE	Low-Density Polyethylene
LSM	Least Squares Mean
Max	Maximum
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
OR	Odds Ratio
OS	Oculus Sinister
OTC	Over The Counter
OU	Oculus Uterque (both eyes)
Paremyd®	1% Hydroxyamphetamine Hydrobromide/ 0.25% Tropicamide
PD	Pupil Diameter
RAF	Royal Air Forc
SAE	Serious Adverse Event
SE	Standard Error
SOC	System Organ Class
SP	Safety Population
TEAE	Treatment Emergent Adverse Event
US	United States
USP	United States Pharmacopeia
VA	Visual Acuity

1. STUDY SUMMARY

Study Number	**OPI-NYXRM-301**
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	2 days, 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 contracti n of the smooth muscle. Phentolamine inhibits contraction of the iris dilator muscle, resulting in a smaller p pil size. Pharmacologically-induced mydriasis is achieved either by stimulating the iris dilator muscle with the use of direct or indirect alpha 1 ag nists (e.g. phenylephrine, hydroxyamphetamine), or by blocking the iris sphincter muscle with the use of muscarinic antagonists or cycloplegic drugs (e.g., tropicamide), or combination (e.g., 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 mydriasis prior to natural reversal. In a reversal of mydriasis phase 2 clinical study (MIRA-1), Nyxol allowed pupil dilation of more subjects to return to baseline faster, it was generally well-tolerated in the eye (most common complaint was mild to moderate conjunctival hyperemia) and there were no clinically meaningful systemic effects as measured by heart rate (HR) and blood pressure (BP).
Study Objectives	The objectives of this study are:

	• To evaluate the efficacy of Nyxol 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 safety of Nyxol • To evaluate any additional benefits of the reversal of pharmacologically-induced mydriasis The Sponsor intends to use this first 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."
Design	A randomized, parallel arm, double-masked, placebo-controlled Phase 3 study in at least 168 randomized subjects (160 evaluable for efficacy), evaluating the safety and efficacy of Nyxol in subjects with pharmacologically-induced mydriasis. Following the successful completion of screening, each subject will be stratified by eye color and then simultaneously be randomized to mydriatic agent (unmasked) and treatment (masked) Treatment randomization will be 1:1, Nyxol or placebo (vehicle). 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, and Paremyd). That is, approximately 60% of the randomized subjects will receive one drop of 2.5% phenylephrine 1 hour before treatment (96 evaluable subjects), approximately 20% will receive one drop of 1% tropicamide 1 hour before treatment (32 evaluable subjects), and approximately 20% will receive Paremyd 1 hour before treatment (32 evaluable subjects). A randomization schema by investigational treatment, mydriatic agent, and iris color is shown in Appendix 2. TREATMENT (NYXOL OR PLACEBO) WILL BE ADMINISTERED TO BOTH EYES (OU). ADULT SUBJECTS (≥ 18 YEARS OLD) WILL HAVE TWO DROPS OF TREATMENT ADMINISTERED FIVE MINUTES APART IN THE STUDY EYE (OD) AND ONE DROP OF TREATMENT ADMINISTERED IN THE NON-STUDY EYE (OS) ONE HOUR

	AFTER MYDRIATIC DRUG INSTILLATION. PEDIATRIC SUBJECTS (< 18 YEARS OLD) WILL HAVE ONE DROP OF TREATMENT ADMINISTERED IN EACH EYE ONE HOUR AFTER MYDRIATIC DRUG INSTILLATION. [redacted]The study and non-study eye will both be evaluated at all assessments.
Patient Population	168 randomized (160 evaluable) healthy subjects.
Inclusion Criteria	1. Males or females ≥ 12 years of age. 2. Otherwise healthy and well controlled subjects. 3. Ability to comply with all protocol-mandated procedures independently and to ttend all scheduled office visits. 4. Adults (≥ 18 years f age) willing to give written informed consent to participate in this study. Children aged 12-17 years to provide igned 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 (e.g., cataract, 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 a) lid scrubs with OTC products (e.g., OCuSOFT® lid scrub, SteriLid®, baby shampoo, etc.) or b) OTC lubricating drops for dry eye (preserved and unpreserved artificial tears), which may have been used prior to, but not at screening until study completion. 6. Recent or current evidence of ocular infection or inflammation in either eye (such as current evidence of clinically significant blepharitis, conjunctivitis, or a history of herpes simplex or herpes zoster keratitis at Screening in either eye). 7. History of diabetic retinopathy or diabetic macular edema.

8. Closed or very narrow angles that in the Investigator's opinion are potentially occludable if the subject's pupil is dilated.
9. History of any traumatic (surgical or nonsurgical) or non-traumatic condition affecting the pupil or iris (e.g., irregularly shaped pupil, neurogenic pupil disorder, iris atrophy, iridotomy, iridectomy, etc.).
10. Known allergy or contraindication to any component of the mydriatic agents or the vehicle formulation.
11. History of cauterization of the punctum or punctal plug (silicone or collagen) insertion or removal.

Systemic:

1. Known hypersensitivity or contraindication to α- and/or β adrenoceptor antagonists (e.g., chronic obstructive pulmonary disease or bronchial asthma; abnormally low blood pressure (BP) or heart rate (HR); second- or third-degree heart blockage or Congestive Heart Failure (CHF); severe diabetes).
2. Clinically s gnificant systemic disease (e.g., uncontrolled diabete myasthenia gravis, cancer, hepatic, renal, endocrine or cardiovascular disorders) that might interfere with the study.
3. 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 (see Appendix 3).
4. Participation in any investigational study within 30 days prior to screening.
5. 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 pre-menstrual, 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.
6. Resting HR outside the normal range (50-110 beats per minute) at the Screening Visit. HR may be repeated only once if outside the normal range following at least a 5-minute rest period in the sitting position.

	7. Hypertension with resting diastolic BP > 105 mmHg or systolic BP > 160 mmHg at the Screening Visit. BP 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]
Number of Investigational Sites	Approximately 12 sites
Estimated Total Sample Size	Approximately 168 randomized healthy subjects, with approximately 160 evaluable subjects.
Sample Size Justification	A sample size of 160 evaluable subjects (80 per treatment group) is needed for the study. The primary efficacy endpoint will be met if subjects show a positive effect for Nyxol ($\alpha = 0.05$ significance, two-tailed). One hundred and sixty evaluable subjects will provide at least [redacted]. All subjects will be [redacted] Furthermore, subjects will be randomized at a

	ratio of 3:1:1 to one of three mydriatic agents (2.5% phenylephrine, 1% tropicamide, and Paremyd). It is assumed that there will be a 5% drop-out during Visit 1. To account for this drop-out, a total of 168 subjects will be randomized into the study.
Primary Efficacy Endpoint	Percentage of subjects' study eyes returning to ≤ 0.2 mm from baseline pupil diameter at 90 minutes.
Secondary Efficacy Endpoints	Secondary efficacy endpoints (for the study eye and the non-study eye) will include: • Percentage of subjects returning to ≤ 0.2 mm from baseline **pupil diameter** at each remaining timepoint (30 minutes, 60 minutes, 2 hours, 3 hours, 4 hours, 6 hours, 24 hours) • Change (in mm) from max **pupil diameter** (0 minutes) at each timepoint (30 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours 4 hours, 6 hours, 24 hours) • Percentage of subjects with unchanged **accommodation** from baseline (-1 hour) at the following timepoints (0 min, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours) • Time (hours) to return to ≤ 0.2 mm from baseline pupil di meter (**time savings analysis**) Measurements: Pupil diameter will be measured with a NeurOptics VIP-300 pupillometer (mm). Accommodation will be measured by the [redacted] The photopic lighting conditions in the room will be defined as [redacted] should be [redacted] for accommodation measurements and the remaining safety assessments (e.g. conjunctival hyperemia, adverse events, 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 timepoints.

	Some of the efficacy endpoints will be analyzed overall, by mydriatic agent, and by light/dark irides at all timepoints. Each mydriatic agent will be analyzed individually, and an additional analysis combining 1% tropicamide and the Paremyd subjects into a "tropicamide" group will be performed. Exploratory analyses may be performed to compare [redacted] t. The Modified Intent-to-Treat (mITT) 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.
Safety Endpoints	The primary safety measures are **conjunctival hyperemia, subjective ocular tolerability, and adverse events** (AEs). Analysis for conjunctival hyperemia includes change from baseline (-1 hour) in the conjunctival hyperemia grading scale (CCLRU images) at each timepoint (0 min, 30 minutes, 60 minutes, 90 minutes, 2 hours, 3, hours, 4 hours, 6 hours, 24 hours), for study eye and non-study eye. Any increase of two units in conjunctival hyperemia between consecutive timepoints is considered an AE. Other safety measures include impairment **in VA (BCDVA and DCNVA) intraocular pressure (IOP), and systemic safety as measured by HR and BP**. Urine pregnancy tests for females of childbear ng potential will be performed at screening. **Subject Questionnaire** values will be summarized by treatment group and timepoint (-1 hour, 0 minutes, 60 minutes, 90 minutes, 3 hours, 6 hours, and 24 hours). Measurements: • Conjunctival hyperemia will be assessed visually with a grading scale (0-3) using images from the CCLRU • Subjective ocular tolerability measured on a 4-point scale (0-3) • BCDVA Distance will be measured binocularly in photopic conditions by Standard ETDRS illuminated chart (on wall or stand) at 4 meters (letters recorded, later converted to logMAR and number of lines) • DCNVA Reading/Near will be measured binocularly in photopic conditions by Near Visual Acuity Chart in the Precision Vision Small 914 Illuminator Cabinet (light box) at 16 inches (~40 cm) (letters recorded, later converted to logMAR and number of lines)

	• IOP is measured with the Tono-Pen • Subject Questionnaire will be a brief symptom survey (Appendix 4)
Study Medications, Dose and Mode of Administration	Nyxol® Eye Drops (Phentolamine Ophthalmic Solution): Two drops (dosed [redacted] apart) of Nyxol in the study eye (OD) (1-hour post mydriatic drug instillation) and one drop of Nyxol in the non-study eye (OS). Note: Pediatric subjects will receive only one drop in each eye. Placebo (Nyxol vehicle): Two drops (dosed [redacted] apart) of Placebo in the study eye (OD) (1-hour post mydriatic drug instillation) and one drop of Placebo in the non-study eye (OS). Note: Pediatric subjects will receive only one drop in each eye.
Duration of Subject Participation and Study	The total length of subject participation is [redacted] [redacted] [redacted] The execution of the entire study (first subject screen through last randomized subject evaluable) is expected to be approximately 3 to 6 months.

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2. INTRODUCTION

2.1. Investigational products

The test product is Nyxol® Eye Drops – 0.75% Phentolamine Ophthalmic Solution (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 and used in place of 1% phentolamine mesylate drug substance, which was how Nyxol had been described in all prior studies. Placebo control is Nyxol vehicle alone.

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) (2020).

Nyxol has been assessed in seven investigator-initiated and sponsored Phase 1 and Phase 2 clinical trials. Across all trials, 168 of 232 adult patients were exposed to at least one dose of phentolamine mesylate ophthalmic solution.

In prior clinical trials, Nyxol has demonstrated a consistent ability to decrease pupil by approximately 20% (~1 – 1.5 mm) in both mesopic and photopic conditions. Key pupil diameter data are summarized below (Table 1).

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

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

Nyxol was observed to be well-tolerated at single doses up to and including 1.0% daily in each eye. This includes 59 patients who received multiple doses of up to 1% Nyxol for at least 14 days. Safety of the patients in these trials was evaluated by AE monitoring, physical examinations, and vital sign assessments. Across all trials, no healthy volunteers or patients reported a treatment-emergent SAE. No deaths occurred in any of the trials. No clinically meaningful changes were observed in physical examinations or vital signs, including blood pressure and heart rate. AEs reported were mild to moderate in intensity with the most common

being transient conjunctival hyperemia and ocular irritation; however, Nyxol dosing at or near bedtime was observed to mitigate or minimize these side effects during the daytime.

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

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

In this study, Nyxol demonstrated a statistically significant reduction in PD from maximum timepoint compared to placebo at 1 hour, 2 hours, 4 hours, and 6 hours. These statistically significant and clinical meaningful (typically greater than 1 mm reduction) PD reductions were observed at each timepoint, including when stratified by both par sympathetic (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 timepoint with estimable results, regardless of whether the PD threshold used was ≤ 0.5 mm above baseline (2-hour timepoint) or the more stringent, ≤ 0 mm above baseline (4-hour and 6-hour timepoints). The reduction in time needed to achieve reversal of mydriasis in either eye w s 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 above baseline. 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 of the planned Ph se 3 primary endpoint (the percent of subjects that returned to within ≤ 0.2 mm of baseline pupil diameter), a statistically significant percentage of subjects in the Nyxol group compared to placebo after being dilated with either phenylephrine or tropicamide returned to baseline at 2 hours (29% vs 13%, respectively; p = .03*)*. This difference widened (68% for Nyxol and 23% for placebo) at 4 hours (p < .0001).

In this study, there were no severe adverse events, with only mild to moderate conjunctival hyperemia that resolved in most patients by 6 hours.

2.3. Design justification

Pupil size is under the control of two 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.[3,4] The radial dilator muscles contain predominantly α-1 adrenergic receptors that can be inhibited by α-1 antagonists[5]; 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 (e.g., phenylephrine, hydroxyamphetamine), by blocking the iris sphincter muscle with the use of muscarinic antagonists or cycloplegic drugs (e.g., tropicamide), or combination (e.g., Paremyd®, which is 1% hydroxyamphetamine hydrobromide and 0.25% tropicamide). A typical induced mydriasis dilates the pupil to 6-8mm,

a size suitable for ophthalmic examination of the retina and other structures of the interior of the eye.

Such pharmacologically induced mydriasis can last from a few hours (typically 6 hours) to days, depending on the pigmentation of the iris, the age of the subject, and other unknown-to-date factors. The side effects of such dilation are sensitivity to light and blurred vision. Also, many dilating agents cause cycloplegia (loss of accommodation), the temporary paralysis of the muscle which allows the eye to focus on near objects. Accelerating mydriatic reversal after an eye exam may be beneficial for many subjects.

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 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 include tropicamide. Tropicamide is a muscarinic antagonist that elicits 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 have material changes in accommodation.

In a previous Phase 2 reversal of pharmacologically induced mydriasis clinical study (MIRA-1), Nyxol was effective at inducing reversal of mydriasis with both adrenergic (phenylephrine) and parasympathetic (tropicamide) mydriatic agents as described above. The placebo outcomes in this study 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 faster for phenylephrine as expected given the pharmacology of Nyxol

Alpha-1 adrenergic antagonists have been shown to be safe and effective for the pharmacological reversal of mydriasis. In 1990, the 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 requiring pupil dilation continue to request an option for rapid reversal of the mydriasis.

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 dose for one drop of Nyxol selected for this study, 0.75%, was based upon: 1) preclinical safety studies, 2) the results of the previous ophthalmic clinical studies described above and in the IB, and 3) clinical studies conducted with varying doses of drugs in the same class.[6]

SUBJECTS ≥ 18 YEARS OLD WILL RECEIVE TWO DROPS (DOSED 5 MINUTES APART) OF TREATMENT (NYXOL OR PLACEBO) IN THEIR STUDY EYE AND ONE DROP OF TREATMENT IN THEIR NON-STUDY EYE. SUBJECT <18 YEARS OLD WILL RECEIVE ONE DROP OF TREATMENT IN THEIR STUDY EYE AND ONE DROP OF TREATMENT IN THEIR NON-STUDY EYE.

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 prior studies, including MIRA-1.

2.5. Compliance

This study will be conducted in compliance with the protocol and 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.

2.6. Study population

A sample size of approximately 168 healthy subjects ≥ 12 years of age will be randomized in a 1:1 ratio to one of two masked treatment arms (Nyxol or Placebo), with the expectation that approximately 160 subjects will complete the study. Randomization will be stratified 1:1 by light/dark color irides and further randomized 3:1:1 to unmasked mydriatic agent (2.5% phenylephrine, 1% tropicamide, and Paremyd). The subjects will be recruited from approximately 12 investigational sites.

3. OBJECTIVES AND PURPOSE

The MIRA-2 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 ag nts with an emphasis on phenylephrine
- To evaluate the efficacy of Nyxol o return subjects to baseline accommodation after worsening (with cycloplegic agents tropicamide and Paremyd)
- To evaluate the safety of Nyxol
- To evaluate any additional benefits of the reversal of pharmacologically-induced mydriasis

The Sponsor intends to use this first 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

4.1. Primary and secondary endpoints

Efficacy:

The primary efficacy endpoint is the percentage of subjects' study eyes returning to ≤ 0.2 mm baseline pupil diameter at 90 minutes.

The study eye is defined as the right eye (OD). The non-study eye is defined as the left eye (OS). The study and non-study eye will both be evaluated at all assessments. Secondary efficacy endpoints (for the study eye and for the non-study eye) will include:

- Percent of subjects returning to ≤ 0.2 mm from baseline **pupil diameter** at each remaining timepoint (30 minutes, 60 minutes, 2 hours, 3 hours, 4 hours, 6 hours, 24 hours)

- Change (in mm) from max **pupil diameter** (0 minutes) at each timepoint (30 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours, 24 hours)
- Percentage of subjects with unchanged **accommodation** from baseline (-1 hour) at each timepoint (0 min, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours)
- Time (hours) to return to ≤ 0.2 mm from baseline pupil diameter (**time savings analysis**)

Measurements:

- Pupil diameter will be measured with a NeurOptics VIP-300 pupillometer (mm).
- Accommodation will be measured by the RAF Near Point Rule (measured in centimeters and then converted to Diopters). Unchanged accommodation from Baseline (-1 hour) is defined as a change from baseline value ≥ -1, as measured in diopters.

[redacted] prior to the pupil diameter, BCDVA and DCNVA (safety measures) at all scheduled timepoints. Subject will sit in the exam chair facing the illuminated chart during the acclimation period and for all assessments. [redacted] for accommodation measurements and the remaining safety assessments (e.g. conjunctival hyperemia, adverse events, 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 timepoints.

Some of the efficacy endpoints will be analyzed overall, by mydriatic agent, and by light/dark irides at all timepoints. Each mydriatic agent will be analyzed individually, and an additional analysis combining 1% tropicamide and the Paremyd subjects into a "tropicamide" group will be performed. Exploratory analyses may be performed to compare efficacy endpoints between the study eye and non-study eye within the same subject.

Safety:

The primary safety measures are conjunctival hyperemia, subjective ocular tolerability, and AEs. Other safety measures include impairment in VA (BCDVA and DCNVA), intraocular pressure (IOP), subject questionnaire, and systemic safety as measured by HR and BP. Urine pregnancy tests for females of childbearing potential will be performed at screening prior to dosing of study medication.

Measurements:

- Conjunctival hyperemia will be assessed visually with a 4-point conjunctival hyperemia grading scale (0-3) with CCLRU images (Appendix 5)
- Subjective ocular tolerability measured on a 4-point scale (0-3)
- BCDVA Distance will be measured binocularly in photopic conditions by Standard ETDRS illuminated chart (on wall or stand) at [redacted] (letters recorded, later converted to logMAR and number of lines)
- DCNVA Reading/Near will be measured binocularly in photopic conditions by Near Visual Acuity Chart in the [redacted] at [redacted] (letters recorded, later converted to logMAR and number of lines)

- IOP is measured with the Tono-Pen
- Subject Questionnaire will be a brief symptom survey

4.2. Description and schedule of visits and procedures

A sample size of approximately 168 healthy subjects ≥12 years of age will be randomized in a 1:1 ratio to one of two treatment arms (Nyxol or Placebo), with the expectation that approximately 160 subjects will complete the study. [redacted] and 3:1:1 by mydriatic agent (2.5% phenylephrine, 1% tropicamide, and Paremyd). Subjects will receive their mydriatic agent 1 hour before treatment. Study procedures are shown in detail in Table 2.

Table 2: Screening and Mydriatic/Treatment Schedule

	Screening	Mydriasis/Treatment	Follow-Up
Day(D)		[redacted]	
Visit			
Hour			
Informed Consent/ Assent			
Screening # assigned			
Med/Ophth History			
Demographics			
Prior/Concomitant Medications*			
Urine Pregnancy Test**			
HR/BP			
[redacted]			
IOP^			
Biomicroscopy			
Ophthalmoscopy^			
Questionnaire^			
Randomization # Assigned			
Treatment: Nyxol/ Placebo			

Screening Visit if subject qualifies becomes the first Treatment Visit. The mydriatic drug(s) will be given at -1 hour.

*Investigators to note changes to concomitant medications at any time throughout the visit.

**UPT is for females of childbearing potential.

^Measurements will be performed as follows:

[redacted]

4.3. Measures taken to minimize/avoid bias

This is a placebo-controlled, double-masked, 1:1 randomized, 2-arm Phase 3 study.

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 alpha-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 isotonic solution
Concentration active	1% – parent 0.75%
Manufacturer drug substance	[redacted]
Manufacturer drug product, placebo	Bioconcept Laboratories Inc.
Storage requirements	[redacted] Stored in a secured location (locked) with no access for unauthorized personnel.

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] (USP) and/or [redacted]l (USP) to [redacted].

4.4.1. Packaging and labeling

The investigational products, active and placebo, are both packaged in 1-ml LDPE dropper bottles containing 0.6 ml solution for single-dose use. Each bottle is wrapped with an

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

4.4.3. Study medication administration

Study medication will be administered by the Investigator or designee at the site on Treatment Visit 1.

4.4.4. Study medication accountability

4.4.4.1. Receipt and disposition of study medication

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

4.4.4.2. Return of study medication

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

4.5. Expected duration of subject participation

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

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

The execution of the entire study (first subject screened through last randomized subject completed) is expected to be approximately 3 to 6 months.

4.6. Randomization and procedure for breaking the code

A randomization code for allocating subjects to treatment will be prepared by a masked biostatistician not connected with the study. Subjects will be randomized by mydriatic agent (unmasked) and stratified by light or dark irides.

At the initiation of study related procedures, every potential subject is assigned a ***Screening number*** in numerical order. Once a subject is qualified for the study, the subject is assigned a ***randomization number*** in the order provided by the biostatistician.

The study medications 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 randomization code be unmasked by the Medical Monitor and made available to the Investigator, Ocuphire, and/or other personnel involved in the monitoring or conduct of this study.

4.7. Collection of data

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

4.8. Completed subject

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

4.9. Non-completing subject

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

4.9.1. Study medication discontinuation

The study medication may be discontinued for the following reasons:

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

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

4.9.2. Reasons for withdrawal from study

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

4.9.3. Entire study terminated

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

4.9.4. Actions after discontinuation

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

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

4.10. Evaluable subjects

Subjects are evaluable for efficacy if they received one or two drops of study treatment and had a pupil diameter measurement at the 90-minute time point at Visit 1.

4.11. Completed study

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

4.12. Procedure after the completion of the study

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

5. SUBJECT INCLUSION AND EXCLUSION CRITERIA

5.1 Subject inclusion criteria

1. Males or females ≥ 12 years of age.
2. Otherwise healthy and well controlled subjects.
3. Ability to comply with all protocol-mandated procedures independently and to attend all scheduled office visits.

4. 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 (e.g., cataract, 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 a) lid scrubs with OTC products (e.g., OCuSOFT® lid scrub, SteriLid®, baby shampoo, etc.) or b) OTC lubricating drops for dry eye (preserved and unpreserved artificial tears), which may have been used prior to, but not at screening until study completion.
6. Recent or current evidence of ocular infection or inflammation in either eye (such as current evidence of clinically significant blepharitis, conjunctivitis, or a history of herpes simplex or herpes zoster keratitis at Screening in either eye).
7. History of diabetic retinopathy or diabetic macular edema.
8. Closed or very narrow angles that in the Investigator's opinion are potentially occludable if the subject's pupil is dilated.
9. History of any traumatic (surgical or nonsurgical) or non-traumatic condition affecting the pupil or iris (e.g., irregularly shaped pupil, neurogenic pupil disorder, iris atrophy, iridotomy, iridectomy, etc.).
10. Known allergy or contraindication to any component of the mydriatic agents or the vehicle formulation.
11. History of cauterization of the punctum or punctal plug (silicone or collagen) insertion or removal.

Systemic:

1. Known hypersensitivity or contraindication to α- and/or β adrenoceptor antagonists (e.g., chronic obstructive pulmonary disease or bronchial asthma; abnormally low blood pressure (BP) or heart rate (HR); second- or third-degree heart blockage or Congestive Heart Failure (CHF); severe diabetes).

2. Clinically significant systemic disease (e.g., uncontrolled diabetes, myasthenia gravis, cancer, hepatic, renal, endocrine or cardiovascular disorders) that might interfere with the study.
3. 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 (see Appendix 3).
4. Participation in any investigational study within 30 days prior to screening.
5. 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 pre-menstrual, 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.
6. Resting HR outside the normal range (50-110 beats per minute) at the Screening Visit. HR may be repeated only once if outside the normal range following at least a 5-minute rest period in the sitting position.
7. Hypertension with resting diastolic BP > 105 mmHg or systolic BP > 160 mmHg at the Screening Visit. BP 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 168 healthy subj cts ≥ 12 years of age will be randomized in a 1:1 ratio to one of two treatment arms (Nyxol or Placebo), with the expectation that approximately 160 subjects will complete the study. 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, and Paremyd). Subjects will receive their mydriatic agent 1 hour before treatment on Treatment Visit 1 on Day 1. There will be no treatment administered on Follow-Up Visit / Day 2.

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 over-the-counter (OTC) ophthalmic medications of any kind within 7 days of screening are prohibited, with the exception of a) lid scrubs with OTC products (e.g., OCuSOFT® lid scrub, SteriLid®, baby shampoo, etc.) or b) OTC lubricating drops for dry eye (preserved and unpreserved artificial tears), which may have been used prior to, but not at screening until study completion. A large number of drugs, both prescription and over-the-counter (OTC), contain active ingredients that can affect PD. This would include many eye drops, such as Visine, that would be used to reduce redness, most cough or cold preparations,

antihistamines and bronchodilators, most nose-drops, most blood-pressure medications, many drugs used for migraines, and many other products.

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

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

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

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

7. ASSESSMENT OF EFFICACY

7.1. Specification of the efficacy parameters

The primary efficacy endpoint is the percentage of subjects' study eyes with two drops of treatment returning to ≤ 0.2 mm baseline pupil diameter at 90 minutes.

The study eye is defined as the right eye (OD). The non-study eye is defined as the left eye (OS). The study and non-study eye will both be evaluated at all assessments. Note that subjects ≥ 18 years old will receive two drops (dosed [redacted]) of treatment in their study eye and one drop of treatment in their non-study eye. Subjects <18 years old will receive one drop of treatment in their study eye and one drop of treatment in their non-study eye. Secondary efficacy endpoints can be found in Section 4.1.

The Modified Intent-to-Treat (mITT) 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.

7.2. Assessing, recording, and analyzing of efficacy parameters

Pupil diameter will be measured at Treatment Visit 1 and Follow-Up Visit 2. Accommodation will be measured at Treatment Visit 1.

- Pupil diameter will be measured with the [redacted] (mm)
- Accommodation will be measured by the [redacted] Unchanged accommodation from Baseline (-1 hour) is defined as a change from baseline value ≥ -1, as measured in diopters.

The photopic lighting conditions in the room will be defined as [redacted]. Subject will sit in the exam chair at a distance of [redacted] from the distance ETDRS [redacted] for all assessments. Subject will be allowed to acclimate to these lighting conditions ([redacted] the pupil diameter, BCDVA and DCNVA (safety measures) at all scheduled timepoints. [redacted] for accommodation measurements and the remaining safety assessments (e.g. conjunctival hyperemia, adverse events, 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 timepoints.

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, where 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 Co sent form is signed, and a Screening number is assigned. Children aged 12-17 years to provide assent. Screening includes an explanation of the study, a medical and ophthalmi history, demographics, a review of prior/concomitant medications a urine pregnancy test (for females of childbearing potential), and HR/BP. The second step in screening includes procedures such as IOP assessment and an ophthalmic examination that includes 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 Screening. 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. As part of the Treatment Visit 1, the subject:

- [redacted]
- [redacted]

- [redacted]

[redacted]. Adult subjects (≥ 18 years old) will have two drops (dosed 5 minutes apart) of treatment administered in the Study Eye (OD) and on drop of treatment administered in the Non-Study Eye (OS) one hour after mydriatic drug instillation. Pediatric subjects (<18 years old) will have one drop of treatment administered in each eye one hour after mydriatic drug instillation.

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 24 hours + 6 hours relative to study treatment administration during Treatment Visit 1 for the following: concomitant medications, HR/BR, pupil diameter, BCDVA, DCNVA, conjunctival hyperemia, AEs, and subject questionnaire.

7.2.4. Unscheduled Visits

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

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

7.2.5. Visit variation

Visits on Day 2 may occur between 24 to 30 hours after the baseline (0 minute) time point on Treatment Day 1.

8. ASSESSMENT OF SAFETY

8.1. Specification of safety parameters

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

- Conjunctival hyperemia measured with CCLRU images using a 4-point scale (0-3) – Appendix 5.
    - 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
- BCDVA Distance will be measured binocularly in photopic conditions by Standard ETDRS [redacted] at [redacted] – Appendix 6
- DCNVA Reading/Near will be measured binocularly in photopic conditions by Near Visual Acuity Chart in the [redacted] – Appendix 6
- IOP is measured with the Tono-Pen
- HR and BP (As per the site's normal equipment and procedures)
- AEs
- Brief Subject Questionnaire – Appendix 4

8.2. Assessing, recording, and analyzing safety parameters

The timing for recording safety parameters may be found in Section 4.2, Table 2: Screening and Mydriatic/Treatment Schedule.

8.3. Adverse events and serious adverse events

All AEs and SAEs that occur following consent and until the final study visit should be collected and recorded on the AE or SAE eCRF page. Only treatment-emergent adverse events/adverse reactions (TEAEs) will be summarized (see Section 9.3.5).

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

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

8.3.1. Adverse event definitions

The following definitions of terms apply to this section:

Adverse event. An AE is any untoward medical occurrence associated with the use of a study medication in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory findings), symptom, or disease temporarily associated with the use of the study medication, whether or not related to the study medication. Study medication includes the investigational drug under evaluation and the comparator product or vehicle placebo that is given or administered during any phase of the study.

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

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

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

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

- Death
- Life threatening adverse event

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

Unexpected adverse event or unexpected suspected adverse reaction. An AE or suspected adverse reaction is considered "unexpected" if i is not listed in the Investigators' Brochure or is not listed at the specificity or severity that has been observed; or, if an Investigators' Brochure 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 Investigators' Brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

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

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

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

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

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

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

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

The term "severe" is used to describe the intensity of an event/reaction; the event/reaction itself may be of relatively minor medical significance (such as a severe headache). This is not the same as a "Serious" Adverse Event, 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: A 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 adverse event.
- Not recovered/not resolved: One of the possible results of an adverse event outcome that indicates that the event has not improved or recuperated.
- Recovered/resolved: One of the possible results of an adverse event outcome that indicates that the event has improved or recuperated.

- Recovered/Resolved with sequelae: One of the possible results of an adverse event 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 adverse event outcome that indicates that the event is improving
- Unknown: Not known, not observed, not recorded, or refused

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

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

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

- **Redness related to instillation that is transient (i. is no longer present 2 hours after instillation) = "conjunctival eryth ma upon instillation"**
- **Redness that is NOT transient (i.e., is present 2 hours after instillation) = "conjunctival hyperemia"**

Expedited reporting of Serious and Unexpected Adverse Events: All SAEs (related and unrelated) will be recorded following subject signature of the informed consent/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 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 th 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 (e.g., 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 US Food and Drug Administration (FDA) on the appropriate schedule depending if the event is drug related or not drug related, expected, unexpected (based on the available information in the Investigators' Brochure).

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.

8.3.2. Follow-up of subjects after adverse events

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

9. STATISTICS

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

9.1. Sample Size

A sample size of 160 evaluable subjects (80 per treatment group) is needed for the study.

The primary efficacy endpoint will be met if subjects show a positive effect for Nyxol (α = 0.05 significance, two-tailed). One hundred and sixty evaluable subjects will provide at least [redacted] The assumptions for this power calculation are estimated from the MIRA-1 Phase 2 results and adjusted for the 3:1:1 mydriatic agent groups.

All subjects will be randomized into the study in a 1:1 ratio to one of the two treatment arms (Nyxol or placebo), with a 1:1 stratification by light/dark irides. Furthermore, subjects will be randomized into the study at a ratio of 3:1:1 to one of the three mydriatic agents, 2.5% phenylephrine, 1% tropicamide, and Paremyd. Therefore, if 96 subjects are randomized to 2.5% phenylephrin then 32 subjects will be assigned to 1% tropicamide and 32 subjects to Paremyd resulting in 160 total subjects.

It is assumed that there will be a 5% drop-out before the 90 minute efficacy assessment. To account for this drop-out, a total of 168 subjects will be randomized into the study.

9.2. Analysis Populations

Modified Intent-to-Treat (mITT): The mITT will include all randomized subjects who received one or two drops of study treatment and then had at least one scheduled pupil diameter measurement during Visit 1. The mITT will be used for the primary endpoint analysis and to analyze selected secondary efficacy endpoints.

Per Protocol Population (PP): The per protocol (PP) population includes all subjects in the mITT who had two drops of study treatment in their Study Eye, had all scheduled pupil diameter measurements during Visit 1, had an increase of > 0.2 mm in PD in the study eye at Time 0 minutes compared to Baseline (Time –1 hour), and had no major protocol deviations. The PP population will be used to analyze selected secondary efficacy endpoints.

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.

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

9.3. Statistical Methods

9.3.1. General considerations

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

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

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

9.3.2. Demographic and baseline characteristics

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

9.3.3. Subject disposition

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

9.3.4. Medical history and prior/co comitant 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 (medicatio s 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.

9.3.5. Analysis of efficacy

Efficacy will be assessed using the mITT and PP with subjects included in the treatment arm in 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 PP 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 timepoint is defined as time 0 minutes, during which maximum pupil diameter is expected.

All efficacy data will be summarized by treatment group, study day and timepoint (-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 baseline pupil diameter at 90 minutes post-treatment in the study eye. 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, 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, with subjects included in their randomized treatment regardless of the treatment they actually received.

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 pupil diameter endpoints are in relation to max (0 minute). Each ANCOVA will be performed using the mITT and PP with subjects included in their randomized treatment regardless of the trea ment they actually received. The output from each ANCOVA will include the LSM and SE for both treatment groups, along with the placebo-corrected LSM, its 95% CI and associated p-value.

A comparison of the study and non-study eye for each subject will be completed for the primary efficacy endpoint, as well as by mydriatic agent.

For each of the secondary endpoints r lated to percent of subjects achieving certain criteria, the analysis will be performed using a logistic regression model with treatment, mydriatic agent, light/dark irides, and the respective baseline as a covariate. For each analysis, the percentage of s bjects in each treatment group meeting the criteria, the OR with 95% CI and p-value will be provided. For these endpoints, the mITT and PP will be used with subjects included in their randomized treatment regardless of the treatment they actually received.

In addition, each secondary efficacy endpoint will be analyzed by light/dark irides 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 the Paremyd subjects into a "tropicamide" 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.

9.3.6. Analysis of safety

No statistical analysis of safety data will be performed in this study. 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.

For HR and BP, Baseline is defined as the screening value. HR and BP values and change from Baseline in the values will be summarized by treatment group and timepoint (screening, 6 hours, and 24 hours).

Observed values and change from baseline (-1 hour) in conjunctival hyperemia at each timepoint (0 min, 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 non-study 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 each timepoint (0 minutes, 90 minutes, 6 hours, and 24 hours), using letters and logMAR units. Letters will be recorded, and later 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. DCNVA 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 non-study eye.

Ocular tolerability values will be summarized by treatment group at timepoint 0 minutes.

Subject Questionnaire values will be summarized by treatment group (overall and by mydriatic agent) and timepoint (-1 hour, 0 minutes, 60 minutes, 90 minutes, 3 hours, 6 hours, and 24 hours).

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

All safety data will be provided in by-subject listings. Safety listings for pediatric subjects may be provided.

9.4. 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.

9.5. 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 (CSR).

10. DIRECT ACCESS TO SOURCE DATA AND DOCUMENTS

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

11. QUALITY CONTROL AND QUALITY ASSURANCE

The progress of the study will be monitored by 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.

12. ETHICAL CONSIDERATIONS AND GCP COMPLIANCE

12.1. GCP compliance

The proposed study is subject to all applicable governmental rules and regulations concerning the conduct of clinical trials on human subjects. This includes, but is not necessarily limited to, the approval of Institutional Review Boards, the Helsinki Declaration, U.S. 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.

12.2. Institutional Review Board (IRB)

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 approv d m terials, all correspondence with the IRB and written approval from the IRB must be made available to Ocuphire, *prior* to the start of subject enrollment into the study.

12.3. Protocol Deviations/Violations

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

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

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

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

Changes implemented without prior approval will be considered Protocol Violations.

12.4. Informed consent and assent requirements

Written informed consent will be obtained from each adult subject. A signed assent form will be obtained for all minors ages 12-17, 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 for 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 inte view, 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 dat 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.

13. DATA HANDLING AND RECORD KEEPING

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

13.1. Data entry

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

13.2. Data quality control and reporting

Data is 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 CRA and site staff. CRAs will review and verify all data collected in the eCRF against source documentation during scheduled monitoring visits. The CRA will work closely with the site staff to address any discrepancies which have been found so that proper resolutions can be made and documented in the clinical database. An audit trail within the system will track all changes made to the data.

13.3. Archiving of data

Archived versions of the database will be saved by Ocuphire consistent with ICH Good Clinical Practices (GCP) Guidelines, complying with whichever of the requirements is longer. Ocuphire will notify the Investigator when documents should be returned.

13.4. Records retention

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

13.5. Amendments to the protocol

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

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

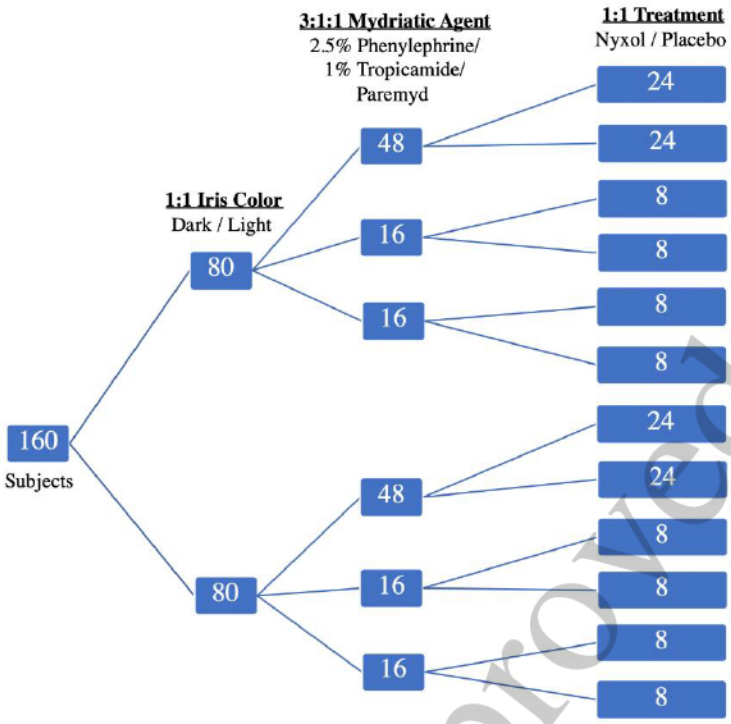
Protocol amendments will be submitted to the appropriate authority(ies) as required by the applicable regulatory requirement(s).

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: RANDOMIZATION SCHEMA FOR EVALUABLE SUBJECTS BY INVESTIGATIONAL TREATMENT, MYDRIATIC AGENT, AND IRIS COLOR



Treatment	Mydriatic Agent	Iris Color
Nyxol	2.5% Phenylephrine	Dark
Placebo	2.5% Phenylephrine	Dark
Nyxol	1% Tropicamide	Dark
Placebo	1% Tropicamide	Dark
Nyxol	Paremyd®	Dark
Placebo	Paremyd®	Dark
Nyxol	2.5% Phenylephrine	Light
Placebo	2.5% Phenylephrine	Light
Nyxol	1% Tropicamide	Light
Placebo	1% Tropicamide	Light
Nyxol	Paremyd®	Light
Placebo	Paremyd®	Light

APPENDIX 3: ADRENERGIC AND CHOLINERGIC DRUGS

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

Alpha-1-agonists	***Non-selective alpha-antagonists***	***Acetylcholine receptor agonists***	***Gastrointestinal***
Methyl norepinephrine	Phenoxybenzamine	Pilocarpine (M_3 receptors)	atropine
Naphazoline	Tolazoline		belladonna
Oxymetazoline	Labetalol		
Tetrahydrozoline	Carvedilol	***Acetylcholine receptor antagonists***	***Parkinsonism***
Phenylephrine			amantadine
Xylometazoline	***Alpha-1-antagonists***		benztropine
	Alfuzosin	Scopolamine	biperiden
Alpha-2-agonists	Prazosin	Dicycloverine	trihexyphenidyl
Brimonidine	Doxazosin	Tolterodine	
Clonidine	Tamsulosin	Oxybutynin	
Guanfacine	Terazosin	Ipratropium	
Guanabenz		Mamba Toxin (MT_7)	
Guanoxabenz	***Alpha-2-antagonists***	Pirenzepine	
Guanethidine	Atipamezole	Telenzepine	
Xylazine	Idazoxan		
Tizanidine	Yohimbine	***Antivertigo***	
Methyldopa		meclizine	
		Scopolamine	

APPENDIX 4: SUBJECT QUESTIONNAIRE

Study: OPI-NYXRM-301 (MIRA-2)
Subject Questionnaire

Subject Number: _____ Visit #___, Date_____

Subject Initials: _____ Assessment timepoint_____

For this questionnaire, if you normally wear glasses to read, put them on now.

1. Are you currently wearing reading glasses? (Check one)

[redacted]

2. Please read the following paragraph without squinting, and then answer the following questions.

Sunset is the time of day when our sky meets the edge 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.

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

[redacted]

3. For the following statements, **circle the number to indicate whether you agree or disagree**.

[redacted]

4. **I am satisfied with my vision at the moment**.

[redacted]

5. **My vision is 100% back to normal at the moment.**

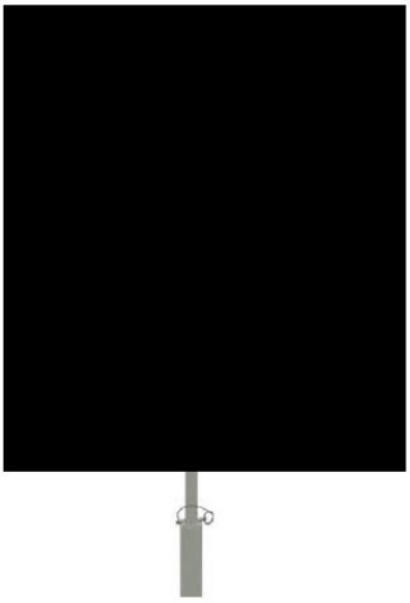
[redacted]

***If Yes**, approximately when did your vision return back to normal?

[redacted]

APPENDIX 5: CONJUNCTIVAL HYPEREMIA GRADING SCALE USING IMAGES FROM CCLRU

APPENDIX 6: VISUAL ACUITY CHARTS



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