CONFIDENTIAL

Clinical Study Protocol
OPI-NYXRMP-303
MIRA-4

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 Pediatric Subjects

[redacted]

Original: **26 October 2021**

SPONSOR SIGNATURE & CONTACTS

Study Title:	Randomized, Parallel-Arm, Double-Masked, Placebo-Controlled Study of the Safety and Efficacy of Nyxol (0.75% Phentolamine Ophthalmic Solution) to Reverse Pharmacologically Induced Mydriasis in Healthy Pediatric Subjects
Study Number:	OPI-NYXRMP-303
Original Protocol:	26 October 2021

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

[redacted]

INVESTIGATOR'S AGREEMENT

OPI-NYXRMP-303
MIRA-4

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 Pediatric Subjects

Original: **26 October 2021**

Investigator Agreement:

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

Signature: _____ Date: _____

Printed Name: _____

PROCEDURES IN CASE OF EMERGENCY

EMERGENCY CONTACT INFORMATION

Role in Study	Name	Contact Information
Clinical Study Leader	[redacted]	[redacted]
Medical Monitor	[redacted]	[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
CI	confidence interval
CRA	clinical research associate
CRO	clinical research organization
°F	degree Fahrenheit
DCNVA	distance-corrected near visual acuity
CRF	case report form
ETDRS	Early Treatment Diabetic Retinopathy Study
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HR	heart rate
IB	Investigators' Brochure
ICH	International Council for Harmonisation
IOP	intraocular pressure
IRB	Institutional Review Board
ITT	Intent-to-Treat
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LSM	least squares mean
MedDRA	Medical Dictionary for Regulatory Activities

mITT	Modified Intent-to-Treat
Nyxol	0.75% Phentolamine Ophthalmic Solution or 1% Phentolamine Mesylate Ophthalmic Solution (Nyxol®)
OD	oculus dexter (right eye)
OR	odds ratio
OS	oculus sinister (left eye)
OTC	over-the-counter
OU	oculus uterque (both eyes)
Paremyd®	1% hydroxyamphetamine hydrobromide/0.25% tropicamide
PD	pupil diameter
PK	pharmacokinetic
PP	Per Protocol
PREA	Pediatric Research Equity Act
RAF	Royal Air Force
SAE	serious adverse event
SAP	Statistical Analysis Plan
SOC	system organ class
SP	Safety Population
TEAE	treatment-emergent adverse event
US	United States
USP	United States Pharmacopeia

1. STUDY SUMMARY

Study Number	**OPI-NYXRMP-303**
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 pediatric 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 acting on adrenergic receptors and is known to inhibit contraction of the iris dilator muscle, resulting in a smaller pupil size. Pharmacologically induced mydriasis is achieved either by stimulating the iris dilator muscle with the use of direct or indirect alpha-1 agonists (eg, phenylephrine, hydroxyamphetamine) or by blocking the iris sphincter muscle with the use of muscarinic antagonists or cycloplegic drugs (eg, tropicamide) or with a combination (eg, Paremyd®, which is 1% hydroxyamphetamine hydrobromide and 0.25% tropicamide). Nyxol, either by directly antagonizing the alpha-1 receptor or by indirectly antagonizing the pupil dilation effect of muscarinic blocking, can expedite the reversal of pharmacologically induced mydriasis prior to natural reversal.
Study Objectives	The objectives of this study are: • To evaluate the safety of Nyxol in pediatric subjects • To evaluate the efficacy of Nyxol to expedite the reversal of pharmacologically induced mydriasis in pediatric subjects The Sponsor intends to use this study to evaluate Nyxol in pediatric subjects aged 3 to 11 for the indication “the treatment of pharmacologically induced mydriasis produced by adrenergic (phenylephrine) or parasympatholytic (tropicamide) agents, or a combination thereof.”
Study Design	This is a randomized, parallel-arm, double-masked, placebo-controlled study in approximately 20 to 30 randomized pediatric subjects evaluating the safety and efficacy of Nyxol in pediatric subjects with pharmacologically induced mydriasis. Pediatric subjects will be recruited for the study into 2 age groups as follows: • 3 to 5 years of age: 10 subjects • 6 to 11 years of age: 10 subjects In this young study population, it is anticipated that some randomized subjects may withdraw. Following the successful completion of screening, each subject will be randomized to unmasked mydriatic agent and masked treatment (refer to

	Appendix 2 for randomization schema). Treatment randomization will be 1:1 (Nyxol or placebo [vehicle]) and will be stratified 1:1 by subject age group (3−5 years of age:6−11 years of age). Mydriatic agent randomization will be 3:1:1 (phenylephrine:tropicamide:Paremyd, respectively). Approximately 60% of the randomized subjects will receive 1 drop of 2.5% phenylephrine in both eyes (OU) 1 hour before treatment (12 subjects), approximately 20% will receive 1 drop of 1% tropicamide OU 1 hour before treatment (4 subjects), and approximately 20% will receive 1 drop of Paremyd OU 1 hour before treatment (4 subjects). Subjects will have 1 drop of masked study treatment (Nyxol or placebo) administered in the study eye (right eye; OD) and 1 drop administered in the fellow eye (left eye; OS). The study eye and the fellow eye will both be evaluated at all assessments unless otherwise specified. The study eye (OD) will always be treated and assessed first. If the subject is not amenable to receiving treatment in the fellow eye (OS), the subject can still participate in the study and only the study eye will undergo study assessments.
Subject Population	Approximately 20 to 30 randomized healthy pediatric subjects, with a target of 20 subjects treated with study medication
Inclusion Criteria	1. Males or premenstrual females 3 to 11 years of age 2. Ability to comply with all protocol-mandated procedures independently and to attend all scheduled office visits 3. Parent/Legal guardian willing to give written informed consent to participate in this study. Children aged 7 to 11 years to provide signed assent form, as well as a separate parental/Legal Guardian consent.
Exclusion Criteria	**Ophthalmic (in either eye):** 1. Clinically significant ocular disease as deemed by the Investigator (eg, amblyopia, congenital cataract, congenital glaucoma) 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 or ocular surgery 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 6. Recent or current evidence of ocular infection or inflammation in either eye (such as current evidence of clinically significant blepharitis, conjunctivitis, or keratitis). Subjects must be symptom free for at least 7 days prior to screening

	7. Closed or very narrow angle that in the Investigator's opinion is potentially occludable if the subject's pupil is dilated 8. History of any traumatic (surgical or nonsurgical) or nontraumatic condition affecting the pupil or iris 9. Known allergy, hypersensitivity, or contraindication to any component of the phentolamine ophthalmic solution or to any component of the mydriatic agents or vehicle formulation **Systemic:** 10. Known hypersensitivity or contraindication to α- and/or β-adrenoceptor antagonists 11. Clinically significant systemic disease (eg, uncontrolled diabetes, cancer, hepatic, renal, endocrine, or cardiovascular disorders) that in the opinion of the Investigator could interfere with the study 12. Subjects with learning disabilities that in the opinion of the Investigator could interfere with the study 13. Initiation of treatment with or any changes to the current dosage, drug, or regimen of any systemic adrenergic or cholinergic drugs within 7 days prior to screening or during the study (Appendix 4) 14. Participation in any investigational study within 30 days prior to screening
Visit Schedule	At Visit 1, subjects will be screened for study eligibility. After screening, eligible subjects will be randomized 3:1:1 to unmasked mydriatic agent (phenylephrine:tropicamide:Paremyd, respectively) and 1:1 to 1 of 2 masked treatment arms (Nyxol or placebo). Treatment randomization will be stratified 1:1 by subject age group (3–5 years of age:6–11 years of age) (Refer to Appendix 2). Each subject will receive 1 drop of mydriatic agent OU 1 hour before study treatment. At Visit 1, measurements as defined in Table 1 will be made before (-1 hour/baseline) and 60 minutes after (max pupil dilation/0 minutes) the mydriatic agent instillation OU (ie, right before the study treatment is administered). Additionally, measurements will be taken at 90 minutes, and 3 hours after study treatment dosing. At Visit 2 (Follow-Up Visit), which is 1 day after Visit 1, measurements as defined in Table 1 will be measured 24 hours after study treatment dosing.
Number of Investigational Sites	Approximately 2 sites
Sample Size Justification	This safety study is not powered for efficacy. In accordance with the Sponsor Pediatric Safety Plan for the indication of reversal of pharmacologically induced mydriasis, the sample size of 20 subjects divided between 2 age groups is adequate to evaluate safety in the pediatric population.

Safety Endpoints	The safety measures are **conjunctival hyperemia, impairment in visual acuity (BCDVA]), vital signs (HR and BP), and adverse events (AEs).** Safety analyses will include: • Change from baseline (-1 hour) in **conjunctival hyperemia** grading (CCLRU images) at each time point (0 minutes, 90 minutes, 3 hours, and 24 hours) for the study eye and fellow eye • Change from baseline (-1 hour) in **BCDVA** at 0 minutes, 90 minutes, 3 hours, and 24 hours for the study eye and fellow eye • Change from screening in **vital signs (HR and BP)** at 3 hours and 24 hours Safety measurements: • Conjunctival hyperemia will be assessed visually [redacted] Appendix 1 • BCDVA will be measured in photopic conditions by a standard Early Treatment Diabetic Retinopathy Study (ETDRS) illuminated chart (on wall or stand) at 4 m (letters recorded). For subjects who are unable to read the letters on a ETDRS chart, the Patti Pics Series ETDRS chart will be used (Appendix 3)
Efficacy Endpoints	Efficacy endpoints for the study eye and fellow eye will include: • Percentage of subjects returning to ≤ 0.2 mm from baseline (-1 hour) photopic **pupil diameter** at each remaining time point (0 minutes, 90 minutes, 3 hours, and 24 hours) • Change (in mm) in photopic **pupil diameter** from max pupil dilation (0 minutes) at each time point (90 minutes, 3 hours, and 24 hours) • Time (hours) to return to ≤ 0.2 mm from baseline (-1 hour) photopic pupil diameter (**time-savings analysis**) Efficacy measurements: Pupil diameter will be measured with a NeurOptics VIP-300 pupillometer (mm) or other similar measurement.
Study Medications, Dose and Mode of Administration	Nyxol® Eye Drops (Phentolamine Ophthalmic Solution): 1 drop OU administered 1 hour post mydriatic drug instillation. Placebo (Nyxol vehicle): 1 drop OU administered 1 hour post mydriatic drug instillation. Study eye will always be treated first. If the subject is not amenable to receiving treatment in the fellow eye (OS) the subject can still participate in the study and only the study eye will undergo study assessments.
Duration of Subject Participation and Study	The total length of subject participation is 2 days, with 2 clinic visits, as summarized below: • Screening/Treatment Visit (Visit 1/Day 1)

	• Follow-Up Visit (Visit 2/Day 2) The execution of the entire study (first subject screened through last randomized subject completed) is expected to be approximately 2 to 4 months.

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2. INTRODUCTION

2.1. Investigational products

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

2.2. Findings from nonclinical and clinical studies

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

Nyxol has been assessed in 9 investigator-initiated and sponsored Phase 1, 2, and 3 clinical trials. Across all trials, 335 of 567 subjects were exposed to at least 1 dose of Nyxol.

[redacted]

Nyxol was observed to be well tolerated in all studies at single doses and multiple daily doses for up to 14 days. Safety of the subjects in these trials was evaluated by adverse event (AE) monitoring, ophthalmic examinations, and vital sign assessments. Across all trials, there were no treatment-related serious adverse events (SAEs). No deaths occurred in any of the trials. No clinically meaningful changes were observed in physical examinations or vital signs, including blood pressure (BP) and heart rate (HR). Adverse events reported were mild to moderate in severity, with the most common being transient conjunctival hyperemia and ocular irritation; however, Nyxol dosing at or near bedtime was observed to mitigate or minimize these side effects during the daytime.

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

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

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

Additionally, Nyxol was effective at returning a statistically significant percent of subjects' eyes to baseline PD compared with placebo treatment at every time point with estimable results, regardless of whether the PD threshold used was ≤ 0.5 mm from baseline (2-hour time point) or the more stringent, ≤ 0 mm from baseline (4-hour and 6-hour time points). [redacted]

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

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

OPI-NYXRM-301 (MIRA-2) was a Phase 3, multicenter, randomized, placebo-controlled, double-masked clinical trial. In MIRA-2, 185 healthy subjects including 14 pediatric subjects aged 12 to 17 from 12 sites were randomized 1:1 to receive Nyxol or placebo (2 drops in the study eye and 1 drop in the fellow eye; pediatric subjects received 1 drop in each eye), 1 hour after pupil dilation with 1 of 3 mydriatic agents (2.5% phenylephrine, 1% tropicamide, or Paremyd). The primary endpoint of an increase in the percent of study eyes returning to baseline PD at 90 minutes after Nyxol compared to placebo was met (Figure 1A [green box]). For subjects in the Modified Intent-to-Treat (mITT) Population treated with Nyxol, 49% had PD return to ≤ 0.2 mm from baseline at 90 minutes compared to only 7% of subjects treated with placebo (p<0.0001). At 60 minutes, 28% of study eyes in subjects who received Nyxol had returned to baseline PD, compared to only 2% of participants who received placebo. The differences in percent of subjects returning to baseline were statistically significant across all time points from 60 minutes through 6 hours (p<0.0001; Figure 1A). Additionally, multiple secondary endpoints met statistical significance, including mean PD, which was significantly smaller with Nyxol compared with placebo at all time points after 30 minutes (p<0.0001; Figure 1B). Finally, the mean time to return to baseline PD for subjects in the Per Protocol (PP) Population was ~2 hours in Nyxol-treated study eyes compared to ~6 hours in placebo-treated study eyes. Within the small pediatric subset, study eyes and non-study eyes treated with 1 drop of Nyxol showed greater reversal of mydriasis compared with eyes treated with 1 drop of placebo from 30 min through 24 hours, although the results were not powered to detect statistically significant differences. Efficacy was demonstrated across the mydriatic agents and for subjects with either light or dark irides.

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

treatment. Nyxol was also well tolerated in the pediatric population, with AEs in a single subject, who reported transient mild pain upon Nyxol instillation OU.

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

2.3. Design justification

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

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

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

In previous reversal of pharmacologically induced mydriasis clinical studies (MIRA-1 and MIRA-2), Nyxol was effective at inducing reversal of mydriasis with both adrenergic (phenylephrine) and parasympathetic (tropicamide) mydriatic agents, as described previously. The placebo outcomes in these studies demonstrated that the natural reversal of mydriasis takes longer with tropicamide than with phenylephrine. Despite this difference, Nyxol was able to

reverse mydriasis faster in the vast majority of eyes regardless of the mydriatic agent used, but it worked faster for phenylephrine (an alpha-1 agonist), as expected given the pharmacology of Nyxol (an alpha-1 and alpha-2 antagonist). A similar pattern of results was seen in the pediatric subjects studied in MIRA-2.

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

The aforementioned completed MIRA-2 study was the first of 2 registration Phase 3 studies evaluating Nyxol for the reversal of pharmacologically induced mydriasis. MIRA-3 the second Phase 3 study, which is similar in design to MIRA-2, is ongoing. This current study, MIRA-4, conducted in pediatric subjects aged 3 to 11 years, supports the Phase 3 Nyxol registration program for the treatment of pharmacologically induced mydriasis using 3 common mydriatic agents (phenylephrine, tropicamide, and Paremyd). This study is primarily for safety and not powered to evaluate efficacy. In accordance with the Sponsor Pediatric Safety Plan for the indication of reversal of pharmacologically induced mydriasis, the sample size of 20 subjects is adequate to evaluate safety in the pediatric population.

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

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

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

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

2.5. Compliance

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

2.6. Study population

Approximately 20 to 30 healthy pediatric subjects 3 to 11 years of age, inclusive, will be randomized in a 1:1 ratio to 1 of 2 masked treatment arms (Nyxol or placebo), to achieve a target of 20 randomized subjects (ages 3−5, n=10; ages 6−11, n=10) who receive treatment with study medication. In this young study population, it is anticipated that some randomized subjects may

withdraw (Section 4.7.1). Treatment randomization will be stratified 1:1 by subject age group (3−5 years of age:6−11 years of age). Mydriatic agent randomization will be 3:1:1 (phenylephrine:tropicamide:Paremyd, respectively). Refer to Appendix 2 for randomization schema. Subjects will be recruited from 2 investigational sites.

3. OBJECTIVES AND PURPOSE

The MIRA-4 study is a randomized, parallel-arm, double-masked, placebo-controlled study of the safety and efficacy of Nyxol (0.75% POS) to reverse pharmacologically induced mydriasis in healthy pediatric subjects.

The objectives of this study are:

- To evaluate the safety of Nyxol in pediatric subjects
- To evaluate the efficacy of Nyxol to expedite the reversal of pharmacologically induced mydriasis in pediatric subjects

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

4. STUDY DESIGN

This is a randomized, parallel-arm, double-masked, placebo-controlled study in approximately 20 to 30 randomized pediatric subjects evaluating the safety and efficacy of Nyxol in pediatric subjects with pharmacologically induced mydriasis. Pediatric subjects will be recruited for the study into 2 age groups as follows:

- 3 to 5 years of age: 10 subjects
- 6 to 11 years of age: 10 subjects

In this young study population, it is anticipated that some randomized subjects may withdraw.

Following the successful completion of screening, each subject will be randomized to unmasked mydriatic agent and masked treatment (refer to Appendix 2 for randomization schema). Treatment randomization will be 1:1 (Nyxol or placebo [vehicle]) and will be stratified 1:1 by subject age group (3−5 years of age:6−11 years of age). Mydriatic agent randomization will be 3:1:1 (phenylephrine:tropicamide:Paremyd, respectively). Approximately 60% of the randomized subjects will receive 1 drop of 2.5% phenylephrine OU 1 hour before treatment (12 subjects), approximately 20% will receive 1 drop of 1% tropicamide OU 1 hour before treatment (4 subjects), and approximately 20% will receive 1 drop of Paremyd OU 1 hour before treatment (4 subjects). Subjects will have 1 drop of masked study treatment (Nyxol or placebo) administered in the study eye (right eye; OD) and 1 drop administered in the fellow eye (left eye; OS). The study eye and the fellow eye will both be evaluated at all assessments unless otherwise specified. The study eye (OD) will always be treated and assessed first. If the subject is not amenable to receiving treatment in the fellow eye (OS), the subject can still participate in the study and only the study eye will undergo study assessments.

4.1. Safety and efficacy endpoints

The study eye is defined as the right eye (OD). The fellow eye is defined as the left eye (OS). The study eye and fellow eye will only be evaluated if mydriatic and study drug were successfully administered. The study eye will always be treated and assessed first.

Safety:

The safety measures are **conjunctival hyperemia, impairment in visual acuity (BCDVA), vital signs (HR and BP), and AEs.**

Safety analyses will include:

- Change from baseline (-1 hour) in **conjunctival hyperemia** grading (Cornea and Contact Lens Research Unit [CCLRU] images) at each time point (0 minutes, 90 minutes, 3 hours, and 24 hours) for the study eye and fellow eye
- Change from baseline (-1 hour) in **BCDVA** at 0 minutes, 90 minutes, 3 hours, and 24 hours for the study eye and fellow eye
- Change from baseline (-1 hour) in **vital signs (HR and BP)** at 3 hours and 24 hours

Safety measurements:

- Conjunctival hyperemia will be assessed visually [redacted]

- Best-corrected distance visual acuity will be measured in photopic conditions by a standard Early Treatment Diabetic Retinopathy Study (ETDRS) illuminated chart (on wall or stand) at 4 m (letters recorded). For subjects who are unable to read the letters on a standard ETDRS chart, [redacted]

Efficacy:

Efficacy endpoints for the study eye and fellow eye will include:

- Percentage of subjects returning to ≤ 0.2 mm from baseline (-1 hour) photopic **PD** at each remaining time point (0 minutes, 90 minutes, 3 hours, and 24 hours)
- Change (in mm) in photopic **PD** from max pupil dilation (0 minutes) at each time point (90 minutes, 3 hours, and 24 hours)
- Time (hours) to return to ≤ 0.2 mm from baseline (-1 hour) photopic PD (**time-savings analysis**)

Efficacy measurements:

- Pupil diameter and pupillary light reflex will be measured with a [redacted] pupillometer (mm) or other similar measurement

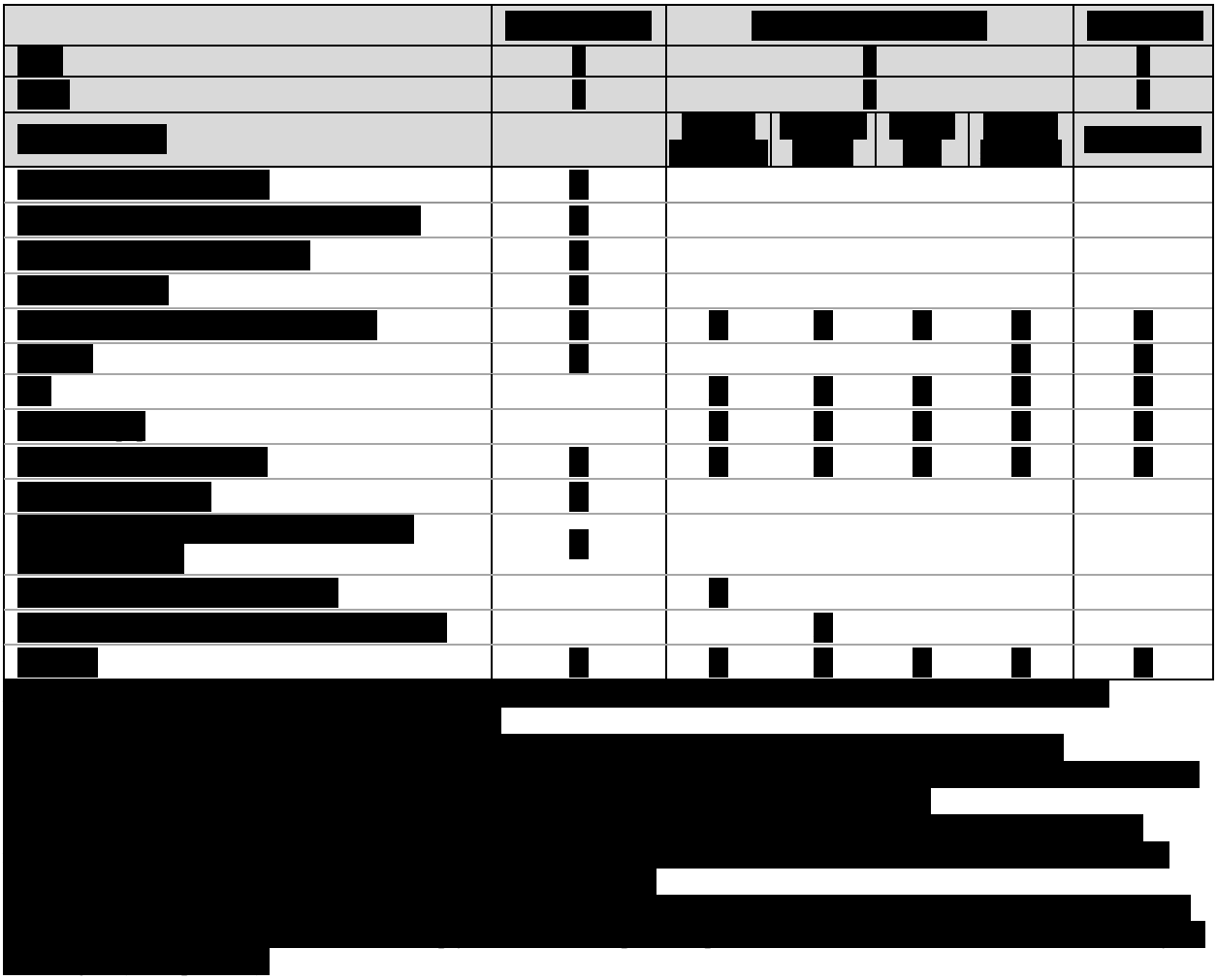
Efficacy endpoints will be summarized by age group and by mydriatic agent but will not be analyzed due to the small sample size for each subgroup. Each mydriatic agent will be summarized individually, and an additional summary combining 1% tropicamide and Paremyd subjects into a "tropicamide or Paremyd" group will be presented. Other subgroups such as sex and race may be summarized as well.

4.2. Description and schedule of visits and procedures

Study procedures are shown in detail in Table 1. For this study, the following time points apply:

- Baseline (-1 hour): just prior to administration of mydriatic agent at -1 hour and time point at which PD measurement is considered normal
- Max pupil dilation (0 minutes): just prior to administration of study medication and time point at which maximum PD is expected

Table 1: Screening and Mydriatic/Treatment Schedule



4.3. Measures taken to minimize/avoid bias

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

4.4. Study medications

Study Medication Identification

Established name	Phentolamine mesylate – parent phentolamine
CAS registry number	65-28-1 – parent 50-60-2
Chemical class	An α-adrenergic antagonist, it is a member of the following classes: imidazoles, of phenols, is a tertiary amino compound and a substituted aniline.
Chemical name	[redacted]
Molecular formula	[redacted]
Molecular weight	[redacted]
Drug name/formulation	Nyxol / aqueous, sterile, non-preserved, isotonic, ophthalmic solution
Concentration active	[redacted]
Manufacturer drug substance	[redacted]
Manufacturer drug product, placebo	[redacted]
Storage requirements	[redacted] per ICH GCP Guidelines.

Formulation

Nyxol (0.75% POS) is [redacted]

4.4.1. Packaging and labeling

The investigational products, active and placebo, are packaged in a 0.5-mL low-density polyethylene Blow-Fill-Seal vial containing [redacted] "Caution – New Drug – Limited by Federal (US) Law to Investigational Use".

4.4.2. Storage of study medication and dispensing

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

4.4.3. Study medication administration

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

4.4.4. Study medication accountability

4.4.4.1. Receipt and disposition of study medication

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

4.4.4.2. Return of study medication

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

4.5. Expected duration of subject participation

The total length of subject participation is 2 days, with 2 clinic visits, as summarized below:

- Screening/Treatment Visit (Visit 1/Day 1)
- Follow-Up Visit (Visit 2/Day 2)

The execution of the entire study (first subject screened through last randomized subject completed) is expected to be approximately 2 to 4 months.

4.6. Randomization and procedure for breaking the code

A randomization code for allocating subjects to treatment will be prepared by an unmasked biostatistician not directly connected with the study. Subjects will be randomized in a 1:1 ratio to 1 of 2 masked treatment arms (Nyxol or placebo). Treatment randomization will be stratified 1:1 by subject age group (3–5 years of age:6–11 years of age). Mydriatic agent randomization will be 3:1:1 (phenylephrine:tropicamide:Paremyd, respectively). Refer to Appendix 2. Note that the mydriatic agent administered is unmasked.

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

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

Subjects may be replaced.

4.7. Collection of data

The source documentation for all data collected in the study will maintained by the Investigator in the subject files at the study site. All original data collected during this trial is to be recorded on paper case report forms (CRFs) and then electronically entered into the database following study completion. The paper CRF is considered to be the source documentation for this study. Data discrepancies will be brought to the attention of the clinical team and investigated by the study monitor and site staff. Study monitors will review data collected in the CRF against any applicable source documentation during remote review or scheduled monitoring visits. The study monitor will work closely with the site staff to address any discrepancies found so that proper resolutions can be made and documented in the clinical database. An audit trail within the system will track all changes made to the data..

Study medication discontinuation

The study medication may be discontinued for the following reasons:

- **Adverse events:** Adverse events include clinically significant laboratory abnormalities and intercurrent diseases reported by the subject or observed by the Investigator with documentation on the CRF
- **Death:** If a subject dies, the AE that caused the death should be documented on the CRF and be noted as serious and fatal
- **Disallowed concurrent medication:** Any medication not allowed by the protocol will be a protocol violation
- **Lack of efficacy:** A subject may elect to discontinue participation in the study for a perceived lack of efficacy

- **Investigator decision:** A subject may be discontinued for reasons other than those bulleted previously if the Investigator thinks it is not in the best interest of the subject to continue
- **Other:** Any other reason for subject discontinuation should be noted on the CRF

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

4.7.1. Reasons for withdrawal from study

- Subject withdraws consent
- Subject is lost to follow-up
- Subject withdraws for other reason
- After being randomized, subject refuses mydriatic dosing
- Subject receives mydriatic agent but refuses to continue with study drug dosing

4.7.2. Subject replacement

Subjects who withdraw from the study may be replaced in an attempt to have the planned number of subjects per age group.

4.7.3.Entire study terminated

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

4.7.4. Actions after discontinuation

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

For any subject who chooses to withdraw consent or who is noncompliant, every possible effort should be made by the Investigator to assure the 3-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.

4.8. Completed study

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

5. SUBJECT INCLUSION AND EXCLUSION CRITERIA

5.1. Subject inclusion criteria

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

1. Males or premenstrual females 3 to 11 years of age
2. Ability to comply with all protocol-mandated procedures independently and to attend all scheduled office visits
3. Parent/Legal guardian willing to give written informed consent to participate in this study. Children aged 7 to 11 years to provide signed assent form, as well as a separate parental/Legal Guardian consent

5.2. Subject exclusion criteria

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

Ophthalmic (in either eye):

1. Clinically significant ocular disease as deemed by the Investigator (eg, amblyopia, congenital cataract, congenital glaucoma) 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 or ocular surgery 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
6. Recent or current evidence of ocular infection or inflammation in either eye (such as current evidence of clinically significant blepharitis, conjunctivitis, or keratitis). Subjects must be symptom free for at least 7 days prior to screening
7. Closed or very narrow angle that in the Investigator's opinion is potentially occludable if the subject's pupil is dilated
8. History of any traumatic (surgical or nonsurgical) or nontraumatic condition affecting the pupil or iris
9. Known allergy, hypersensitivity, or contraindication to any component of the phentolamine ophthalmic solution or to any component of the mydriatic agents or vehicle formulation

Systemic:

10. Known hypersensitivity or contraindication to α- and/or β-adrenoceptor antagonists
11. Clinically significant systemic disease (eg, uncontrolled diabetes, cancer, hepatic, renal, endocrine, or cardiovascular disorders) that might interfere with the study
12. Subjects with learning disabilities that in the opinion of the Investigator could interfere with the study

13. Initiation of treatment with or any changes to the current dosage, drug, or regimen of any systemic adrenergic or cholinergic drugs within 7 days prior to screening or during the study (Appendix 4)
14. Participation in any investigational study within 30 days prior to screening

6. TREATMENT OF SUBJECTS

Approximately 20 to 30 healthy pediatric subjects 3 to 11 years of age, inclusive, will be randomized in a 1:1 ratio to 1 of 2 masked treatment arms (Nyxol or placebo), to achieve a target of 20 randomized subjects (ages 3−5, n=10; ages 6−11, n=10) who receive treatment with study medication. In this young study population, it is anticipated that some randomized subjects may withdraw (Section 4.7.1). Treatment randomization will be stratified 1:1 by subject age group (3−5 years of age:6−11 years of age). Mydriatic agent randomization will be 3:1:1 (phenylephrine:tropicamide:Paremyd, respectively). Refer to Appendix 2 for stratification schema. Subjects will receive their mydriatic agent OU 1 hour before treatment with study medication on the Treatment Visit (Visit 1/Day 1). There will be no treatment administered on the Follow-Up Visit (Visit 2/Day 2).

The study eye (OD) will always be treated and assessed first. If the subject is not amenable to receiving treatment in the fellow eye (OS), the subject can still participate in the study and only the study eye will undergo study assessments.

6.1. Treatment adherence

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

6.2. Concomitant medications

As noted in the exclusion criteria (Section 5.2), use of any topical prescription or OTC ophthalmic medications of any kind within 7 days of screening is prohibited.

Additionally, initiation of treatment with or any changes to the current dosage, drug, or regimen of any systemic adrenergic or cholinergic drugs (Appendix 4) 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 CRF. Investigators are encouraged to contact the Medical Monitor for any questions regarding allowed medications. Judgment of continued study participation by the subject and inclusion of this subject's subsequent visits in the safety and efficacy analysis will be made by Ocuphire.

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

7. ASSESSMENTS OF SAFETY AND EFFICACY

7.1. Specification of safety parameters

Safety and efficacy endpoints are detailed in Section 4.1.

The assessment of safety and tolerability will be performed by:

- Conjunctival hyperemia measured [redacted]
  - [redacted]
  - [redacted]
  - [redacted]
  - [redacted]
- Best-corrected distance visual acuity will be measured in photopic conditions by a standard ETDRS illuminated chart (on wall or stand) at [redacted] (letters recorded)
- Heart rate and BP (as per the site's normal equipment and procedures)
- Adverse events (refer to Section 7.3 for AE and SAE assessment)

The efficacy assessment, PD, will be measured as follows:

- Pupil diameter and pupil reactivity will be measured [redacted] or other similar measurement

7.2. Assessing, recording, and analyzing safety and efficacy parameters

The photopic lighting conditions in the room will be defined [redacted] ETDRS illuminated chart (on wall or stand) [redacted] Room lights should be turned on for the remaining safety assessments (eg, conjunctival hyperemia, AEs, and BP/HR). The subject will be in the same room for all assessments, and every effort will be made to have the same person perform the measurements at all time points.

7.2.1.Screening Visit (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 the Treatment Visit, when the dose of study treatment is given.

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

Children aged 7 to 11 years must provide assent. Screening includes an explanation of the study, a medical and ophthalmic history, demographics, a review of prior/concomitant medications, and HR/BP assessment. The second step in screening includes an ophthalmic examination consisting of biomicroscopy and external examinations.

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

7.2.2. Treatment Visit (Visit 1/Day 1)

Treatment Visit should be the same day as the Screening Visit. Once the subject has completed the screening assessments part of the visit and it is confirmed that he/she meets all of the inclusion criteria but none of the exclusion criteria, the visit will then transition to the Treatment Visit assessments. The study eye is OD and the fellow eye is OS. The study eye (OD) will always be treated before the fellow eye (OS). If the subject is not amenable to receiving treatment in the fellow eye (OS), the subject can still participate in the study and only the study eye will undergo study assessments.

As part of the Treatment Visit, the subject will:

- Be randomized to 1 of 2 treatment arms, with further randomization by mydriatic agent and stratification by age group
- Receive 1 of 3 approved mydriatic agents (2.5% phenylephrine, 1% tropicamide, or Paremyd) at -1 hour (baseline). The mydriatic agent will be administered as a single drop in each eye. If a drop of mydriatic agent is missed, the Investigator should give the drop again. Note: Mydriatic agent is administered ***after*** assessments conducted at -1 hour (baseline)
- Receive the masked study medication (Nyxol or placebo) based on his/her randomized treatment arm (kit assignment) at 0 minutes (max pupil dilation). Note: Study medication is administered ***after*** assessments conducted at 0 minutes (max pupil dilation). Study medication will be administered OU 1 hour after mydriatic drug instillation. Subjects will receive 1 drop in the study eye (OD) and 1 drop in the fellow eye (OS). If a drop of study medication is missed, the Investigator should give the drop again. The study eye (OD) will always be treated and assessed first. If the subject is not amenable to receiving treatment in the fellow eye (OS), the subject can still participate in the study and only the study eye (OD) will undergo study assessments
- Be assessed at -1 hour (baseline), 0 minutes (max pupil dilation), 90 minutes, and 3 hours relative to study treatment for the following: PD (OD, OS), BCDVA (OD, OS), and conjunctival hyperemia (OD, OS), as well as concomitant medications and any AEs
- Be assessed at -3 hours, relative to study treatment for HR/BP

7.2.3.Follow-Up Visit/Day 2

On Day 2, the subject will return to the clinic for the 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 the Treatment Visit for the following: concomitant medications, HR/BP, PD, BCDVA, conjunctival hyperemia, and AEs.

7.2.4. Unscheduled Visits

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

As noted in Section 4.7.4, 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 (-1 hour time point on Treatment Day 1).

7.3. 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 CRF page. Only treatment-emergent adverse events (TEAEs)/adverse reactions will be summarized (Section 8.3.5).

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

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

7.3.1. 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 temporally associated with the use of the study medication, whether or not related to the study medication. Study medication includes the investigational drug under evaluation and the comparator product or vehicle placebo that is given or administered during any phase of the study.

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

The occurrence of AEs should be sought by open-ended questioning of the subject at each visit during the study. At each clinic assessment/visit, study personnel should ask the following

question: “Have you had any problems since your last assessment/visit?”. Adverse events also may be detected when they are volunteered by the subject during or between visits or through study assessments.

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

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

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

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

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

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

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

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

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

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

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

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

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

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

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

Action taken in response to an AE is coded as:

- Dose increased: An indication that a medication schedule was modified by addition; either by changing the frequency, strength, or amount
- Dose not changed: An indication that a medication schedule was maintained
- Dose reduced: An indication that a medication schedule was modified by subtraction, either by changing the frequency, strength, or amount

- Dose interrupted: An indication that a medication schedule was modified by temporarily terminating a prescribed regimen of medication
- Drug withdrawn: An indication that a medication schedule was modified through termination of a prescribed regimen of medication
- Not applicable: Determination of a value is not relevant in the current context
- Unknown: Not known, not observed, not recorded, or refused

Additional other action taken:

- Concomitant medication
- Hospitalization

Outcome of an AE is coded as:

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

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

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

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

Expedited reporting of Serious and Unexpected Adverse Events: All SAEs (related and unrelated) will be recorded following subject signature of the informed consent/assent and until the Follow-Up Visit (Visit 2/Day 2). Any SAEs "suspected" to be related to the study medication and discovered by the Investigator at any time **after** the study should be reported.

Any SAE that occurs must be reported to the clinical research organization (CRO) within 24 hours of its occurrence or within 24 hours of learning of its occurrence. Recurrent episodes, complications, or progression of the initial SAE must be reported to the CRO as follow-up to the original episode within 24 hours of the Investigator receiving the information. Information about

all SAEs will be collected and recorded on the SAE form. All pertinent medical records and information collected during the treatment and follow-up of the subject should be maintained at the site with a copy emailed to MIRA4_Safety@Oculoscr.com. The Investigator must assess the SAE relationship and complete the SAE form. The CRO may request additional information. Follow-up information (eg, discharge summary) will be retained in the subject's chart and a copy will be emailed to MIRA4_Safety@Oculoscr.com. In addition, all SAEs should be recorded on the AE CRF page with the serious question marked "Yes".

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

Any death occurring during the study or 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 Visit, that is not reasonably associated with study medication administration, does not require completion of the SAE form.

7.3.2. 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.

8. STATISTICS

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

8.1. Sample size

[redacted]

Appendix 2

8.2. Analysis populations

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

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

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

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

8.3. Statistical methods

8.3.1. General considerations

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

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

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

8.3.2. Demographic and baseline characteristics

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

8.3.3. Subject disposition

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

8.3.4. Medical history and prior/concomitant medications

Medical history will be coded using the latest version of Medical Dictionary for Regulatory Activities (MedDRA) and will be summarized by treatment group using the SP.

Prior medications (medications with an end date prior to the date of randomization) and concomitant medications (medications with a start or end date after the date of randomization) will be coded using WHODrug and will be summarized by treatment group using the SP.

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

8.3.5. Analysis of safety

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

Safety endpoints are indicated in Section 4.1.

For HR and BP, baseline is defined as the screening value. Heart rate and BP values and change from baseline in the values will be summarized by treatment group and time point (screening, 3 hours, and 24 hours).

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

The visual acuity assessments (BCDVA) will be summarized for the study eye and fellow eye at time points 0 minutes, 90 minutes, 3 hours, and 24 hours using the number of letters correctly identified. Letters will be recorded and may later be converted to logMAR by programming for such analyses. One letter is equivalent to 0.02 logMAR. As a reference, 5 letters are represented by 1 line. Treatments will be compared using the same ANCOVA model proposed for the continuous efficacy endpoints. Verbatim descriptions of AEs will be coded using MedDRA. Only TEAEs (those that occur after the first dose of study medication or increasing in severity after initiation of study medication) will be summarized. Treatment-emergent AEs and SAEs will be summarized by treatment group, by system organ class (SOC), severity, and relationship to study medication. Deaths, withdrawal from study medication due to AEs, and withdrawal from the study due to AEs will each be summarized by treatment group. Note that in MedDRA, ocular events are coded to the SOC of "special senses". Thus, using SOC in the summaries will provide a separation of ocular and non-ocular AEs.

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

8.3.6.Analysis of efficacy

Efficacy will be assessed using the mITT and PP Populations, with subjects included in the treatment arm into which they were randomized. Confirmatory analyses may be performed using the ARP. Generally, for the analysis of the efficacy endpoints, only observed case data will be used.

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

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

[redacted]

change (in mm) in photopic from max (0 minutes)

[redacted]

[redacted]

In addition, efficacy endpoints will be summarized by age group and by mydriatic agent but will not be analyzed due to the small sample size for each subgroup. Each mydriatic agent will be summarized individually, and an additional summary combining 1% tropicamide and Paremyd subjects into a "tropicamide or Paremyd" group will be presented. Other subgroups, such as light/dark irides, sex, and race, may be summarized as well.

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

Imputation will be performed for missing data in limited circumstances as described in the SAP. For the summarization and analysis of efficacy endpoints and safety data, observed case data generally will be used.

8.5. Procedure for reporting deviations from the statistical plan

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

9. 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.

10. 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 CRFs, subject records (source documents), signed consent/assent forms, records of study medication receipt, storage, preparation, and disposition, and regulatory files related to this study.

11. ETHICAL CONSIDERATIONS AND GOOD CLINICAL PRACTICE COMPLIANCE

11.1. Good Clinical Practice compliance

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

11.2. Institutional Review Board

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

11.3. Protocol deviations/violations

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

A reportable protocol deviation is defined as nonadherence to the protocol that involves inclusion/exclusion criteria, affects subject safety, rights, or welfare, or has the potential to affect the integrity of the data. Examples of major protocol deviations include study enrollment by ineligible subject, loss of key data such as equipment malfunction (eg, pupillometer), and/or use of a prohibited medication during the study. **However, treatment in study eye only and missed assessments as outlined in this protocol will not be considered protocol deviations.**

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

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

Changes implemented without prior approval will be considered protocol violations.

11.4. Informed consent and assent requirements

Written informed consent will be obtained from the legal guardian of each subject. A signed assent form will be obtained for all minors ages 7 to 11, as well as a separate parental/Legal Guardian consent. A copy of the signed and dated consent/assent document will be given to each subject or parent guardian. The original signed and dated informed consent/assent document must be maintained in the study files at the Investigator's site.

The Investigator is responsible for ensuring that no subject is subject to any study-related examination or activity before that subject has given informed consent/assent. The subject must

give written consent/assent after the receipt of detailed information. The verbal explanation will cover all the elements specified in the written information provided to the subject.

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

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

A copy of the signed and dated consent/assent document will be given to each subject/legal guardian. 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.

12. DATA HANDLING AND RECORD KEEPING

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

12.1. Data entry

Study-specific data that have been outlined in the protocol will be entered into the clinical database by the Data Manager.

12.2. Data quality control and reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database. Query reports pertaining to data omissions and discrepancies will be forwarded to the Clinical Investigator and Ocuphire, or its designee, for resolution. The study database will be updated in accordance with the resolved query reports. All changes to the study database will be documented.

12.3. Archiving of data

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

12.4. Records retention

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

12.5. Amendments to the protocol

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

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

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

13. BIBLIOGRAPHY

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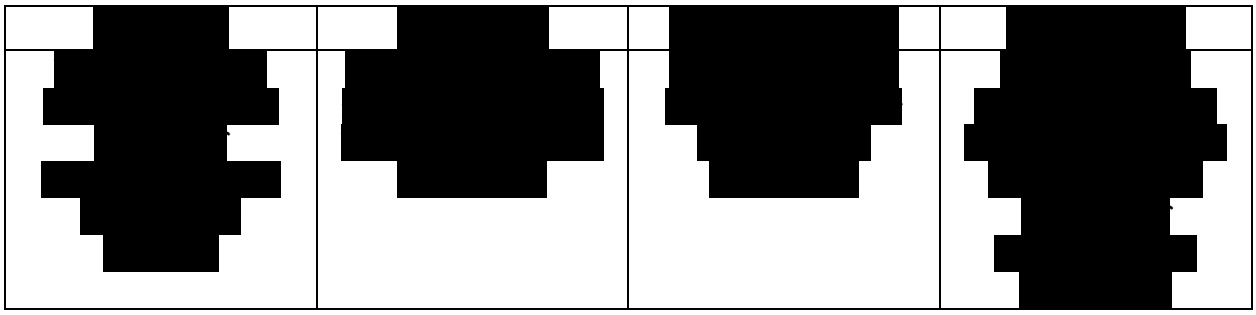
APPENDIX 1: CONJUNCTIVAL HYPEREMIA GRADING SCALE USING IMAGES FROM CCLRU



APPENDIX 2: RANDOMIZATION SCHEMA BY AGE GROUP, MYDRIATIC AGENT AND INVESTIGATIONAL TREATMENT

All subjects	1:1 age group	3:1:1 mydriatic agent	1:1 treatment
20	10 3-5 years of age	[redacted]	[redacted]
		[redacted]	[redacted]
		[redacted]	[redacted]
	10 6-11 years of age	[redacted]	[redacted]
		[redacted]	[redacted]
		[redacted]	[redacted]

APPENDIX 3: [redacted] ETDRS CHART

[redacted]

APPENDIX 4: 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.***