

Clinical Research Protocol
RANDOMIZED TRIAL TO EVALUATE THE EFFICACY OF A SMALL VOLUME
TOPICAL OPHTHALMIC SPRAY FOR PUPILLARY DILATION IN CHILDREN

Protocol Number:	20-32530A
NCT Number:	NCT06366217
Version Date:	1.0
Intervention:	MydCombi® (Eyenovia, Inc., New York City, NY)
IND Number:	N/A
Development Phase:	N/A
Sponsor:	N/A
Funding Organization:	Partial funding from the NIH-NEI EY002162 Core Grant for Vision Research and Eyenvia Inc.
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Coordinating Center:	N/A

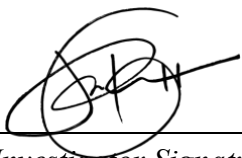
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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: 20-32530

Protocol Title: RANDOMIZED TRIAL TO EVALUATE THE EFFICACY OF A SMALL VOLUME TOPICAL OPHTHALMIC SPRAY FOR PUPILLARY DILATION IN CHILDREN



Investigator Signature

1 August 2024

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LIST OF ABBREVIATIONS

μL	microliter
AE	adverse event
CFR	Code of Federal Regulations
CRF	case report form
D	diopter
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	informed consent form
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
PI	Principal Investigator
SAE	serious adverse experience
SE	spherical equivalent
SOC	standard of care

PROTOCOL SYNOPSIS

TITLE	RANDOMIZED TRIAL TO EVALUATE THE EFFICACY OF A SMALL VOLUME TOPICAL OPHTHALMIC SPRAY FOR PUPILLARY DILATION IN CHILDREN
SPONSOR	N/A
FUNDING ORGANIZATION	Partial funding from the NIH-NEI EY002162 Core Grant for Vision Research and Eyenovia Inc.
NUMBER OF SITES	1
RATIONALE	Topical ophthalmic medications in the form of eye drops are widely used in pediatric ophthalmology clinics to achieve pupillary dilation and cycloplegia. The standard of care eye drops typically deliver more medication than can be absorbed by the human eye, contributing to waste. This study aims to directly compare the effect of a small volume ophthalmic spray with standard of care eye drops on pupillary dilation and cycloplegia among pediatric patients.
STUDY DESIGN	This is a randomized, unblinded, non-inferiority study.
PRIMARY OBJECTIVE	To interpret a non-inferiority analysis of using the MydCombi device for pupillary dilation in pediatric patients.
SECONDARY OBJECTIVE	The secondary objectives are to assess changes in IOP, measures of acceptance of delivery modality, and cycloplegia in pediatric patients.
NUMBER OF SUBJECTS	66
SUBJECT SELECTION CRITERIA	<u>Inclusion Criteria:</u> Children ≤ 18 years of age who were having pupillary dilation performed as part of their routine eye examinations at the UCSF Pediatric Ophthalmology Clinic. <u>Exclusion Criteria:</u> Inability to cooperate with study interventions (eye drop administration, pupillometry, autorefraction); medication allergy to tropicamide, or phenylephrine; congenital or iatrogenic anterior segment abnormalities; anisocoria; not attending the scheduled appointment; or use of pupil altering topical or systemic medications.
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	1% tropicamide, 2.5% phenylephrine at 8 μL dose Product will be administered twice by a 1 metered spray using the fixed dosed MydCombi® device.

CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	1% tropicamide at (approximately 50 µL) dose 2.5% phenylephrine at (approximately 50 µL) µL dose Product will be administered once with one drop of each of the above, topically through standard-sized eyedrop bottles (standard of care).
DURATION OF SUBJECT PARTICIPATION	Participants will be on study for one clinic visit, approximately 40 minutes – 90 minutes.
CONCOMITANT MEDICATIONS	N/A
EFFICACY EVALUATIONS	N/A
PRIMARY OUTCOME(S)	Pupillary dilation, as assessed by: • Maximum pupil diameter after dilation • Change in pupil constriction percentage
SECONDARY ENDPOINTS	• Intraocular pressure after dilation • Measure of acceptance of delivery modality • Cycloplegia (Intra-eye change in spherical equivalent from before and after dilation)
OTHER EVALUATIONS	N/A
SAFETY EVALUATIONS	N/A
PLANNED INTERIM ANALYSES	An interim analysis is planned after the enrollment of 20 participants for data collection quality.
STATISTICS Primary Analysis Plan	A non-inferiority analysis was done on the primary outcomes with pre-dilation measurements and treatment arm used as predictor variable and participants treated as random effect variable. A non-inferiority margin of 0.4 mm in maximum pupillary dilation will be used to determine non-inferiority.
Rationale for Number of Subjects	Sample size was determined based on a power calculation to provide a power of 0.905 assuming a non-inferiority margin of 0.4 mm in pupillary dilation with a standard deviation of 0.55 mm.

1 BACKGROUND

The current mydriatic eye drops employed in eye examinations face several constraints, including the risk of cross-contamination and challenges in administering to patients with restricted mobility. Notably, higher doses of phenylephrine, a common component, have been associated with significant systemic cardiovascular side effects. Administering smaller-volume eye drops offers several benefits, such as extending the lifespan of medication bottles and thus decreasing the economic burden of medication and lowering the risk of systemic absorption and local side effects. In this study, we assess the efficacy of a small new volume ophthalmic spray, MydCombi in a pediatric population. At a horizontal 1-metered spray, MydCombi dispenses 8 μ L of medication for pediatric pupillary dilation and cycloplegia, with two sprays recommended per dose.

1.1 Overview of Non-Clinical Studies

Previous studies have determined much medication waste can be attributed to the fact that many patients often miss their eye on self-administration attempts. In specific, a prior study that for every drop that makes it into a glaucoma patient's eye, seven drops are wasted during the instillation attempt (Robin A et al., *Rev Ophthalmol.* 2010; Stone J et al., *Arch Ophthalmol.* 2009). Further, since prescription eye drop bottles elute drops that exceed the capacity of the human eye, approximately 80% of their medication to wasted overflow and/or systematic absorption (Farkouh A et al., *Clin Ophthalmol.*, 2016).

1.2 Overview of Clinical Studies

Previous work comparing 20 μ L drops and 50 μ L drops of 0.5% pilocarpine hydrochloride produced an equivalent miotic response in healthy volunteers, establishing that smaller volumes of eye drops can be equally efficacious (File R et al., *Arch Ophthalmol.*, 1980). Additionally, it was noted that the 20 μ L drops of the 0.5% drug solution produced fewer local symptoms (i.e., transient blurring of vision, some stinging upon administration, watering eyes, and mild redness) than the larger 50 μ L drops. Of note, it has also been previously established that in the pediatric population, administering 10 μ L eye drops using a small volume applicator produced similar efficacy to standard of care (Hoppe C et al., *Ophthalmol.*, 2023).

2 STUDY RATIONALE

It will be useful to understand the efficacy of the MydCombi device on pupillary dilation in pediatric patients.

2.1 Risk / Benefit Assessment

N/A

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective is to conduct an interpret a non-inferiority analysis of using the MydCombi device for pupillary dilation in a cohort of pediatric patients.

3.2 Secondary Objectives

The secondary objectives are to assess changes in IOP, measures of acceptance of delivery modality, and cycloplegia in pediatric patients.

4 STUDY DESIGN

4.1 Study Overview

This is a single-center, unblinded, randomized, simple, 1:1 trial. 132 eyes of 66 patients are planned. Each participant will have baseline autorefraction, pupillometry, and intraocular pressure measured at baseline. Each participant will be administered medication in each eye for pupillary dilation. One eye will receive a single drop of 1% tropicamide and 2.5% phenylephrine, and the other eye will receive 1% tropicamide and 2.5% phenylephrine using the MydCombi spray. 30 minutes after medication administration, each participant will have refraction, pupillometry, and intraocular pressure measured again.

Participants who meet all inclusion criteria and none of the exclusion criteria will be enrolled in the study.

The total duration of participation for the individual subject will be 40 minutes – 90 minutes. The total duration of the study is expected to be 1 month.

4.2 Primary Efficacy Outcome

The primary outcome is pupillary dilation as assessed by: 1) maximum pupil diameter after dilation, and 2) change in pupil constriction percentage.

4.3 Secondary Efficacy Outcome

The secondary outcomes are as follows:

- 1) Intraocular pressure after dilation.
- 2) Measure of acceptance of delivery modality (assessed by questionnaire).
- 3) Cycloplegia (defined as intra-eye change in spherical equivalent from before and after dilation).

4.4 Safety Evaluations

We do not expect there to be additional safety evaluations related to this trial.

5 SUBJECT SELECTION

5.1 Study Population

Pediatric patients who were scheduled for routine pupillary dilation at the University of California, San Francisco Pediatric Ophthalmology clinic are eligible.

5.2 Inclusion Criteria

1. Male or female ≤ 18 years of age
2. Scheduled for routine pupillary dilation

3. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative and ability for subject to comply with the requirements of the study.

5.3 Exclusion criteria

1. Inability to cooperate with study interventions (eye drop administration, pupillometry, autorefraction)
2. Medication allergy to tropicamide or phenylephrine
3. Congenital or iatrogenic anterior segment abnormalities
4. Anisocoria
5. Not attending the scheduled appointment
6. Use of pupil altering topical or systemic medications
7. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data.

6 CONCURRENT MEDICATIONS

N/A

6.1 Allowed Medications and Treatments

N/A

7 STUDY TREATMENTS

7.1 Method of Assigning Subjects to Treatment Groups

66 eligible patients will be randomly assigned receive treatment using MydCombi in either the right or left eye, and standard of care drops in the other eye. Treatment groups (standard of care or MydCombi) will be randomized in a 1:1 ratio using a web-based computer-generated randomization.

7.2 Blinding

Due to the nature of the study, investigators, research staff, and patients will be unblinded to the intervention.

7.3 Formulation of Test and Control Products

The intervention product is the MydCombi spray (Eyenovia, Inc., New York City, NY). MydCombi is a colorless, topical ophthalmic spray containing tropicamide 1% and phenylephrine hydrochloride 2.5%. Each metered spray delivers 0.008 mL which contains 0.08 mg tropicamide and 0.25 mg phenylephrine hydrochloride. Two sprays are administered per dose. The MydCombi dispenser is composed of the two parts: the cartridge, which holds the drug solution, and the base, which provides the power for dispensation. The control products are two eye drop formulations: 1% tropicamide, and 2.5% phenylephrine. A single eye drop of each product is administered per dose.

7.4 Supply of Mydcombi at the Site

A supply of MydCombi (commercial product) will be provided by the manufacturer to the site for use in this study. The dedicated study Mydcombi will be in the locked clinic office in the locked materials cabinet. Study staff will transport study devices to and from clinic rooms for use.

7.4.1 Administration Instructions

MydCombi dispensers will be charged, assembled, and inspected for any damage or cracks prior to use in the study. Verify that the device has not have low battery using the battery light (red light indicates low charge) and that the dispenser is ready (blinking blue light means the device needs to be reset). Wipe the device with a 70% isopropyl alcohol (IPA) wipe and wait 3 minutes. To load the drug in the dispenser, hold the device upright and press the fill button on the device until the upper mist light turns blue. Then slowly release the fill button over 3 seconds. Ensure that the fill button has returned to position, if not, repeat with a new cartridge. To verify that the device is working correctly, do a test mist spray away any participants. To administer the drug, aim MydCombi dispenser to the center of patient's eye and confirm that blue mirror on the device is centered on the cornea. Press and release Mist button on the device to deliver the drug. Two sprays will be administered as described in the Mydcombi label. The individual administering the drug will view the patient from the side to ensure that administrations are delivered accurately. If an administration is not delivered accurately, it will be repeated and this will be recorded.

Administer the control drops by gently squeezing eye drop bottles to elute one drop of medication into the patient's open eye. Repeat for all topical ophthalmic medications denoted in this protocol.

7.4.2 Storage

MydCombi dispensers and cartridges should be stored by the study site at controlled room temperature.

8 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject or subject's legal representative. If appropriate, assent must also be obtained prior to conducting any study-related activities.

8.1 Clinical Assessments

8.1.1 Demographics

Demographic information (date of birth, gender, eye color) will be recorded at Visit 1.

8.1.2 Baseline measurements

Baseline spherical power, cylindrical power, axis; maximum pupil diameter, minimum pupil diameter, pupil constriction percentage, pupil construction latency, and intraocular pressure will be recorded at Visit 1.

8.1.3 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop and times), outcome, treatment and relation to study intervention will be recorded in the notes section of the electronic data collection form.

9 EVALUATIONS BY VISIT

9.1 Visit 1

1. Review the study with the subject (subject's legal representative) and obtain electronic informed consent and HIPAA authorization and assent, if appropriate.
2. Assign the subject a unique screening number.
3. Record demographics data.
4. Record non-cycloplegic autorefraction, pupillometry, and tonometry.
5. Randomize MydCombi to be given to right or left eye.
6. Administer dilating eye drops.
7. Record cycloplegic autorefraction, pupillometry, tonometry, and acceptability.

9.2 Early Withdrawal

1. Record any Adverse Experiences and record reason for withdrawal.

10 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

10.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign, symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in Table 1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 2.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

10.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

10.2.1 Serious Adverse Experience Reporting

Study sites will document all SAEs that occur (whether or not related to study drug) per [UCSF CHR Guidelines](#). The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB), the site investigator will report SAEs to the IRB.

11 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

11.1 Early Discontinuation of Study Drug

A subject may be discontinued from study treatment at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Subject withdrawal of consent (or assent)
- Subject is not compliant with study procedures
- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment
- Protocol violation requiring discontinuation of study treatment
- Lost to follow-up
- Sponsor request for early termination of study

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue study treatment should come in for an early discontinuation visit as soon as possible and then should be encouraged to complete all remaining scheduled visits and procedures.

All subjects are free to withdraw from participation

12 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject, study staff, or investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Inappropriate administration of medication (too many eye drops)
- Failure to measure or record data

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The study investigator will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor representative and the Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files.

13 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

13.1 Data Sets Analyzed

All eligible patients who are randomized into the study and receive at least one dose of the study drug will be included in the analysis.

13.2 Demographic and Baseline Characteristics

The following demographic variables at screening will be summarized: gender, age, noncycloplegic autorefraction, and tonometry.

13.3 Analysis of Primary Endpoint

Measurement of changes in pupillary dilation will be computed by subtracting post-dilation values from baseline measurements. Inter-eye differences will be determined by comparing the change in standard of care scores to MydCombi scores. A 95% confidence interval will be applied, with a non-inferiority threshold set as described above. Analysis of covariance will be conducted post-dilation, with pre-dilation measurements serving as covariates, treatment arms as predictors, and participants as random effects. Non-inferiority for each outcome will be assessed based on whether the regression coefficient's 95% confidence interval overlaps with the non-inferiority threshold. Wald P values will be computed to test if the regression coefficient exceeds the non-inferiority threshold. Each patient's eyes will be analyzed separately, and data will be entered into REDCap (Vanderbilt University) and analyzed using R.

13.4 Analysis of Secondary Endpoints

1) Intraocular pressure after dilation: Measurement of changes in intraocular pressure will be computed by subtracting post-dilation values from baseline measurements. Inter-eye differences will be determined by comparing the change in standard of care scores to MydCombi scores. Analysis of covariance will be conducted post-dilation, with pre-dilation measurements serving as covariates, treatment arms as predictors, and participants as random effects.

2) Measure of acceptance of delivery modality: Data from acceptance questionnaire (Appendix 2) will be analyzed as follows: we will calculate mean, median, and standard deviation for discomfort ratings (questions 1 and 2) and compute frequencies and percentages for preference choices (question 3). Paired t-test will be used to compare discomfort ratings between eye drops and spray. Chi-square test will be used to analyze preference distribution. We will perform a Spearman's correlation between discomfort ratings and dilation preferences.

3) Cycloplegia (defined as intra-eye change in spherical equivalent from before and after dilation): Measurement of changes in spherical equivalent will be computed by subtracting post-dilation values from baseline measurements. Inter-eye differences will be determined by comparing the change in standard of care scores to MydCombi scores.

13.5 Interim Analysis

An interim analysis is planned after the enrollment of 20 participants for data collection quality.

13.6 Sample Size and Randomization

Sample size was determined based on a power calculation to provide a power of 0.905 assuming a non-inferiority margin of 0.4 mm in pupillary dilation with a standard deviation of 0.55 mm.

DATA COLLECTION, RETENTION AND MONITORING

13.7 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF) OR paper CRF when the information corresponding to that visit is available. Subjects will not be identified by name in the study database, but will be identified by a site number, subject number, and birthdate.

For eCRFs: If a correction is required for an eCRF, the time and date stamps track the person entering or updating eCRF data and creates an electronic audit trail. *For paper CRFs*: If a correction is made on a CRF, the study staff member will line through the incorrect data, write in the correct data and initial and date the change.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. A copy of the CRF will remain at the Investigator's site at the completion of the study.

13.8 Data Management Procedures

The data will be entered into a validated database. The Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

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

13.9 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 on a regular basis. Queries are entered, tracked, and resolved through the EDC system directly. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

13.10 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database. At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

13.11 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years following marketing of the investigational product or for two years after centers have been notified that the IND has been discontinued. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

13.12 Monitoring

Monitoring during the study will be performed by the principal investigator.

13.13 Subject Confidentiality

In order to maintain subject confidentiality, only a site number, subject number and subject birthdate will identify all study subjects on CRFs and other documentation submitted to the Sponsor. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

14 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

14.1 Protocol Amendments

Any amendment to the protocol will be written by the study investigator. Protocol amendments cannot be implemented without prior written IRB approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

14.2 Institutional Review Board

The protocol and consent form will be reviewed and approved by the IRB of the participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB in accordance with the standard operating procedures and policies of the IRB, and the Investigator will keep the IRB informed as to the progress of the study. The Investigator will obtain assurance of IRB compliance with regulations.

Any documents that the IRB may need to fulfill its responsibilities will be submitted to the IRB. The IRB's written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. Informed consent modifications or changes may not be initiated without prior written IRB approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may

affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

14.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will send an IRB-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

14.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

14.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.

6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

REFERENCES

Farkouh A, Frigo P, Czejka M. Systemic side effects of eye drops: a pharmacokinetic perspective. Clin Ophthalmol. 2016;10:2433–2441.

File RR, Patton TF. Topically applied pilocarpine: human pupillary response as a function of drop size. Archives of Ophthalmology. 1980;98:112-5.

Hoppe CB, Yonamine S, Kao BW, Turner ML, Ou Y, Han Y, Keenan JD, Oatts JT. Randomized Trial to evaluate the efficacy of the nanodropper device for pupillary dilation and cycloplegia in children. Ophthalmology. 2023;130:324-30.

Robin AL, Covert D. Beyond compliance: Getting the drops in. Rev Ophthalmol. 2010;19:71-3.

Stone JL, Robin AL, Novack GD, Covert DW, Cagle GD. An objective evaluation of eyedrop instillation in patients with glaucoma. Archives of ophthalmology. 2009;127:732-6.

APPENDIX 1. STUDY VISIT SCHEDULE

	VISIT 1
Informed Consent	X
Demographics	X
Non-cycloplegic autorefraction	X
Non-cycloplegic pupillometry	X
Non-cycloplegic tonometry	X
Eye dilation using standard of care drops	X
Eye dilation using MydCombi	X
Cycloplegic autorefraction	X
Cycloplegic pupillometry	X
Cycloplegic tonometry	X
Measure of acceptability	X
Adverse Experiences	X

APPENDIX 2. ACCEPTANCE QUESTIONNAIRE

1. On a scale of 0 to 10 with 0 being absolutely no discomfort and 10 being the most uncomfortable you can imagine, how would you rate having your eye dilated with normal eye drops? _____

2. On a scale of 0 to 10 with 0 being absolutely no discomfort and 10 being the most uncomfortable you can imagine, how would you rate having your eye dilated with the spray (MydCombi)? _____

3. If you could choose to have your eyes dilated with normal eye drops or the spray (MydCombi), which would you choose:

- a. Normal eye drops
- b. Spray (MydCombi)
- c. No preference