Excelencia en oftálmicos

Study Protocol:

SOPH185-0521/I

Title: Phase I clinical study to evaluate the safety and tolerability of PRO-185 ophthalmic solution applied in clinically healthy subjects

Sponsor: Laboratorios Sophia, S.A. de C.V.
Version Date: July 27, 2022
Version: 2.0

This document was prepared in accordance with FDA, ICH, and GCP guidelines.

Change History

Sections, paragraphs and subsections	Main modifications made
Cover Page Headers and Footers	Updated version number, version date, and page count.
Change History	Added Change History to describe the major changes between this version and the previous one.
Content	Section page numbers have been changed, and new subsections have been added.
Index of tables, graphs and figures	The page numbers of tables and figures were modified, and the table of contents of graphs was added.
Study leaders	The Study Director's affiliation was changed from "Medical Manager" to "Regional Clinical Research Manager."
List of abbreviations	The abbreviation TRPL is being dropped. Tear film break-up time.
1.1 Synopsis	The protocol version number and version date have been changed. Hypothesis H_0 changed to: "PRO-185 ophthalmic solution is safe and tolerable for ophthalmic use, with any of the following adverse events occurring in 20% or less of subjects: increased intraocular pressure (IOP >5 mmHg), heart rate changes (>15 bpm), increased systemic blood pressure [>15 mmHg systolic (SBP) or >10 mmHg diastolic (DBP)], pharmacological mydriasis, or grade 3 and 4 conjunctival hyperemia." The underlined words "any of" were added to highlight that only one of the adverse events described was necessary. It is described that comparisons will be between pre- and post-treatment measurements at visits 1 and 2 and between measurements at the pre-PT baseline visit and measurements at the final visit. In Statistical Methodology, the Kolmogorov-Smirnov test and the Mann-Whitney U test were changed to the Wilcoxon signed-rank test; the Pearson X^2 *test was also changed to the binomial* X^2, *and the* McNemar test was withdrawn .
2.2.1 Naphazoline	Typo fixed.
2.2.3 Menthol	Typographical and writing errors were corrected. Added information on menthol irritability and toxicity testing. Added information on the usefulness of using menthol.
2.3 Background on the research product	It was added that a preclinical safety and toxicity study of the PRO-185 ophthalmic solution has already been carried out.
2.3.1 Preclinical safety and toxicity study of PRO-185 solution	New section describing the comparative preclinical safety and toxicity study of PRO-185 ophthalmic solution versus a marketed product, Nazil® Contamination . The study involved administering the drugs four times daily for 30 days to New Zealand albino rabbits. The results show no relevant safety and toxicity data for any of the investigational products. There are also no clinical changes or manifestations demonstrating the unsafety or toxicity of the PRO-185 formulation. Therefore, it is concluded that the product is safe and nontoxic for ocular use in the selected animal model.

2.3.2 Preclinical study of an ophthalmic solution of naphazoline 0.1% and hypromellose 0.5% (higher concentration of the main components of PRO-185)	Information was added about another preclinical study different from the one already described.
2.5.1 Known potential risks	Typos were fixed. Information on the safety of menthol as an excipient is added to the concentration found in the PRO-185 solution.
2.7 Justification of the study	Information related to the preclinical study of PRO-185 is added, as well as the safety of menthol and its use at the concentration found in the PRO-185 solution.
3.2 Hypothesis	or "any of" are added to specify that only one of the adverse events described in the hypothesis must occur at the frequency also described. It is specified that comparisons will be made between pre- and post-treatment measurements from visits 1 and 2, and between measurements from the pre-PT baseline visit and measurements from the final visit.
Table iii . Operational definition of variables in 7.4.4 Definition of variables, methods and scales for their measurement	The Statistical Tests of all variables in the table are modified.
7.4.5.7 SICCA ocular surface staining	The statement is removed: After evaluating the TRPL, between 4 and 8 minutes after instillation of the dye.
10.1.1 Calculation methodology	Typo corrected.
10.1.2 Size calculation	Typographical errors are corrected. The equations for calculating the sample size are modified. Absolute values are added in parentheses to the description of the signs.
10.2 Clinical data management	Typo corrected.
10.3 Statistical methodology	The previous version specifies that the statistical analysis will be performed using SPSS version 19.0. The new version specifies that either SPSS or R software can be used, and that the available version will be used.
10.3.2.1 Analysis for primary variables	The Kolmogorov-Smirnov, Mann-Whitney U, and Pearson X2 tests are removed · *The* Wilcoxon signed-rank and Binomial *X2 tests are added* · It is specified that there will be a comparison between pre- and post-treatment measurements for visits 1 and 2; and another comparison between baseline (pre-PT) visit measurements and final visit measurements.
Literature	Bibliographies were added and the location of the bibliographic references in the list was modified, following the order of appearance in the protocol.

Content

Index of tables, graphs and figures

Study leaders

The administrative structure of the sponsoring party, corresponding to Laboratorios Sophia, SA de CV, is shown in Table 1. Study managers .

Table i. Study leaders

Function	Name/Contact	Membership ¥
Medical Director of the study	[redacted]	Medical Director
Director of the study		Regional Medical Affairs Manager
Operations Manager		Regional Clinical Research Manager
Author of the Protocol		Medical Editor
Biostatistics		Biostatistics Manager

Signature page

From the sponsor

Name:	
	Signature
Qualification:	
	Date

Name:	
	Signature
Qualification:	
	Date

Name:	
Qualification:	**Signature**
	Date

Name:	
Qualification:	**Signature**
	Date

Researcher Agreement

I agree to conduct this clinical study according to the design and guidelines of this protocol, adhering to its provisions. I declare that I will conduct the study in accordance with the standards of Good Clinical Practice and will report all information and data as outlined in the protocol, particularly any adverse events. I will also manage clinical supplies provided by the sponsor strictly in accordance with this protocol. I understand that the information that identifies me may be used by the sponsor. Because the information contained in this protocol and the Investigator's Manual is confidential, I understand that sharing it with any third party not involved in the approval, supervision, or conduct of the study is prohibited. I will ensure that necessary precautions are taken to protect the information from loss, inadvertent disclosure, or access by unauthorized third parties.

Name: *[Write the researcher's full name* **Qualification:** Principal Investigator **Name of the center:** [*Write name of study center]* **Geographic location (city/state/country)** *[Write the geographic data of the center]*	**Signature** **Date**

List of abbreviations

Ad	Adherence
cAMP	cyclic adenosine monophosphate
AO	Both eyes
av	Visual acuity
AVMC	Best corrected visual acuity
BPC	Good clinical practices
CDM	Clinical Data Management
CEI	Research Ethics Committee
CI	Investigation Committee
COFEPRIS	Federal Commission for the Protection against Sanitary Risks
CTO	Ocular staining grading
OF	Standard deviation
IUD	Intrauterine device
EA	Adverse event
eCFR	*Electronic* Case *Form Report*)
ECG	Electrocardiogram
EEP	Punctate epithelial erosions
USA	United States of America
FC	Heart rate
FCI	Informed consent form
FR	Respiratory rate
H_0	Null hypothesis
H_1	Alternative hypothesis
ICH	International Council on Harmonization (*International Council for Harmonization*)
ICMJE	*International Committee* of Medical Journal Editors (ICME) *of Medical Journal Editors*)
IP	Principal Investigator
IP_3	Inositol triphosphate
ITT	By intention to treat (ITT *) to Treat*)
bpm	Beats per minute
mmHg	Millimeters of mercury
NOM	Mexican Official Standard

WHO	World Health Organization
PAD	Diastolic blood pressure
PI	Research product
PIO	Intraocular pressure
PNA	Unanticipated problems
Pre-TP	Prior to applying TP Ofteno ®
Post-Tx	Post-treatment
PP	Population by protocol
Post -TP	After application of TP Ofteno ®
Pre -Tx	Pre-treatment
RAM	Adverse drug reaction
RNEC	National Registry of Clinical Trials
SDV	*Source* Document Verification *Document Verification*)
SICCA	*Sjögren 's International* Collaborative *Clinical Alliance*)
SPSS	Statistical Package for the Social Sciences (*Statistical Package for the Social Sciences) Package for the Social Sciences*)
SRAM	Suspected adverse drug reaction
TEROS 40	Tear evaporation rate at 40% ambient humidity (*Tear Evaporation Ocular Surface Rate*)
TF	Fluorescein staining
TVL	Lissamine green staining
TRPM8	Cation receptor potential channel subfamily 8 (*Transient Receptor Potential) Cation Channel Subfamily M Member 8*), also known as the cold-sensing cation channel.
UFTLS	Pharmacovigilance and Technovigilance Unit of Sophia Laboratories
VV	Current version
X^2	Chi-square
°C	Degrees Celsius

1. Summary of the protocol

1.1 Synopsis

<table>
<tr><td colspan="2">Title of the study:

Phase I clinical study to evaluate the safety and tolerability of PRO-185 ophthalmic solution applied in clinically healthy subjects</td></tr>
<tr><td>Study number:
SOPH185-0521/I</td><td>Creation date:
05/17/2021</td></tr>
<tr><td>Protocol version:
2.0</td><td>Version date:
27/07/2022</td></tr>
<tr><td>Therapeutic indication:
Ocular vasoconstrictor</td><td>Use:
Conjunctival hyperemia</td></tr>
<tr><td>Estimated duration of the study (from the first visit of the first patient to the preparation of the final report) :
5 months</td><td>Clinical development phase:
Yo</td></tr>
<tr><td colspan="2">Goals:

Main objective:

• To evaluate the safety and tolerability of PRO-185 ophthalmic solution in ophthalmologically and clinically healthy subjects.

Specific objectives:

• To evaluate the safety of PRO-185 ophthalmic solution by measuring the incidence of subjects experiencing an increase of >5 mmHg in intraocular pressure 20 minutes after application compared to the initial value.

• To evaluate the safety of PRO-185 ophthalmic solution by measuring the incidence of subjects experiencing a heart rate variation of >15 beats per minute 20 minutes after application.

• To evaluate the safety of PRO-185 ophthalmic solution by measuring the incidence of subjects with an increase in systolic blood pressure of >15 mmHg or >10 mmHg in diastolic blood pressure 20 minutes after application.</td></tr>
</table>

- To assess tolerability by the incidence of grade 3 and 4 conjunctival hyperemia.
- To assess tolerability by the incidence of pharmacological mydriasis.
- Evaluate tolerability by the incidence of expected and unexpected adverse events.

Hypothesis :

H $_0$ = PRO-185 ophthalmic solution is safe and tolerable for ophthalmic use, as it occurred in 20% or less of subjects with any of the following adverse events: increased intraocular pressure (IOP >5 mmHg), heart rate variation (>15 bpm), increased systemic blood pressure [>15 mmHg systolic (SBP) or >10 mmHg diastolic (DBP)], pharmacological mydriasis, or grade 3 and 4 conjunctival hyperemia.

$$H_0: p - p_0 \leq \delta$$

H $_1$ = PRO-185 ophthalmic solution is not safe or tolerable for ophthalmic use because more than 20% of subjects experienced any of the following adverse events: increased IOP (>5 mmHg), heart rate variation (>15 bpm), increased systemic blood pressure [>15 mmHg systolic (SBP) or >10 mmHg diastolic (DBP)], pharmacological mydriasis, or grade 3 and 4 conjunctival hyperemia.

$$H_1: p - p_0 > \delta$$

Comparisons will be made between pre- and post-treatment measurements from visits 1 and 2, and between measurements from the baseline visit (before PT application) and measurements from the final visit. Only one of the adverse event values described in the hypothesis need be abnormal with a frequency >20% between the comparisons (pre-treatment with post-treatment; or pre-PT baseline with values from the final visit) for the null hypothesis to be rejected.

Study design:

Phase I clinical study, controlled, non-comparative, open, single-center

Number of subjects (planned and analyzed):

Number of subjects planned: 22 subjects.

Diagnosis and main inclusion criteria:

- Ophthalmologically and clinically healthy subjects.

Selection criteria:

Inclusion criteria:

- Be clinically healthy.

- Have the ability to voluntarily grant signed informed consent.
- Be able and willing to comply with scheduled visits, treatment plan, and other study procedures.
- Be between 18 and 45 years old.
- Women of childbearing potential must ensure continued use (started ≥ 30 days prior to signing the ICF) of a hormonal contraceptive method or intrauterine device (IUD) during the study period.
- Have a best-corrected visual acuity of 20/30 or better in both eyes.
- Have vital signs within normal parameters.
- Have an intraocular pressure ≥10 and ≤ 21 mmHg.

Exclusion criteria:

- Be a user of topical ophthalmic products of any kind.
- Being allergic to naphazoline or having a history of intolerance to nasal decongestants or ocular vasoconstrictors.
- Have a history of a suspected diagnosis of primary angle closure, primary angle closure, or angle-closure glaucoma.
- Having iridotomies or waiting for iridotomies.
- Have conjunctival hyperemia grade 3 and 4 on the Efron scale.
- Have ocular surface staining with a value equal to or greater than 3 on the SICCA scale in either eye.
- Being a user of medications or herbal products, by any other route of administration.
- For women: be pregnant, breastfeeding, or planning to become pregnant during the study period.
- Having participated in clinical research studies 90 days prior to inclusion in this study.
- Having previously participated in this same study.
- Be a contact lens user who cannot discontinue use during the study.
- Having a history of any chronic-degenerative disease, including diabetes and high blood pressure.
- Present inflammatory or infectious disease, active at the time of entering the study.
- Present unresolved injuries or traumas at the time of entering the study.
- Have a history of any type of eye surgery.
- Having undergone non-ophthalmological surgical procedures in the last 3 months.

Research Product (RP):

- PRO-185. Naphazoline 0.03% and hypromellose 0.2%. Solución oftálmica. Laboratorios Sophia, S.A. de C. V., Zapopan, Jalisco, Mexico.
- Dosage : 1 drop 4 times a day in both eyes (AO).
- Route of administration: Ophthalmic.

Duration of treatment:	**Subject's duration in the study:**
8 days	10 days

Evaluation criteria:

Primary outcome variables

- Intraocular pressure
- Heart rate
- Systemic blood pressure
- Pupillary diameter
- Conjunctival hyperemia

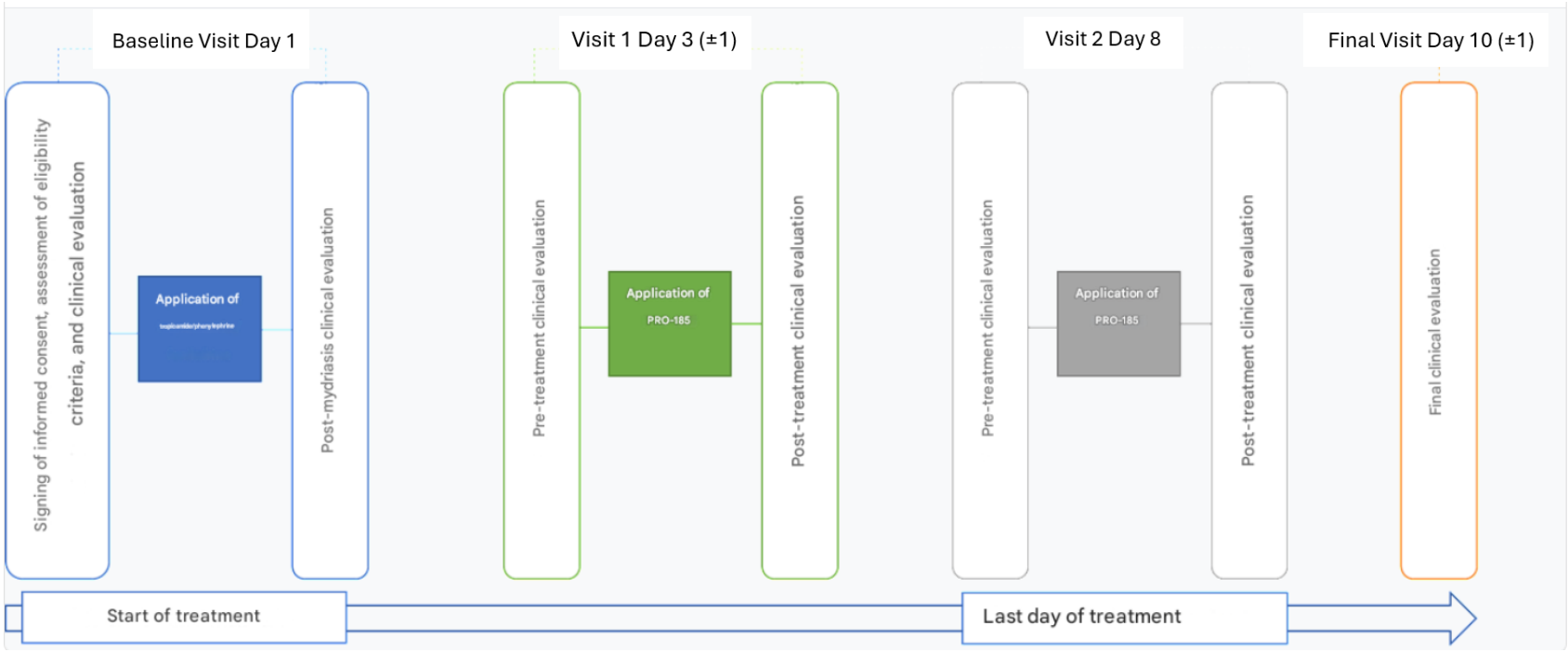
Secondary outcome variables:

- Best corrected visual acuity
- Changes in ocular surface staining
- Incidence of chemosis
- Incidence of expected adverse events
- Incidence of unexpected adverse events

Statistical methodology

Statistical analysis will be performed using specialized statistical software (SPSS statistical package, available version, or R software). Quantitative variables will be expressed as measures of central tendency: mean, median, standard deviation, and ranges. Qualitative variables will be presented as frequencies and percentages. Statistical analysis to rule out differences will be performed using nonparametric statistics, using the Wilcoxon signed-rank test for one sample, for quantitative variables. Analysis between qualitative variables will be performed using the binomial X^2 (Chi-square) test or Fisher's Exact test (when applicable). An alpha (α) ≤ 0.05 will be considered significant .

1.2 Study diagram



1.3 Subject's activity schedule

Procedures	*Baseline visit* *Day 1*		*Visit 1* *Day 3 (+1)*		*Visit 2* *Day 8*		*Final visit* *Day 10 (+1)*
	Pre-TP	Post - TP	Pre - Tx	Post- Tx	Pre - Tx	Post- Tx	Without treatment
FCI Signature	X						
Medical record	X						
Eligibility criteria	X						
Subject code assignment	X						
Adverse events	X	X	X	X	X	X	X
Heart rate	X	X	X	X	X	X	X
Respiratory rate	X		X		X		X
Blood pressure	X	X	X	X	X	X	X
Temperature (°C)	X		X		X		X
Urine pregnancy test	X						X
AVMC	X		X		X		X
Anterior segment evaluation	X	X	X	X	X	X	X
Pupillary diameter measurement	X	X	X	X	X	X	X
Eye stains	X	X	X	X	X	X	X
Intraocular pressure	X	X	X	X	X	X	X
Gonioscopy	X						
Pharmacological mydriasis (phenylephrine/tropicamide)	X						
Evaluation of the posterior segment	X		X		X		X
Application of PRO-185 during a visit			X		X		
Delivery of material for the subject		X					
Delivery of the study medication		X					

Procedures	*Baseline visit*		*Visit 1*		*Visit 2*		*Final visit*
	Day 1		*Day 3 (+1)*		*Day 8*		*Day 10 (+1)*
	Pre-TP	Post - TP	Pre - Tx	Post- Tx	Pre - Tx	Post- Tx	Without treatment
Evaluation of concomitant medications	X		X		X		X
Adherence assessment			X		X		
Return of study medication						X	
Withdrawal from the subject's diary						X	

PRE-TP: prior to application of 0.8% tropicamide and 5% phenylephrine ophthalmic solution (TP Ofteno®, indicates that the checks will be performed prior to pharmacological mydriasis. Post-TP: post application of the TP Ofteno® solution, indicates that the checks will be performed with pharmacological mydriasis. Pre- Tx : pretreatment, indicates the checks to be performed before the application of the investigational medicinal product. Post-Tx : post-treatment, indicates the evaluations to be performed within 20-40 minutes after the application of the investigational medicinal product. ICF: informed consent form. BCVA: best corrected visual acuity.

2. Introduction and background

2.1 Theoretical framework

2.1.1 The red eye

Red eye is a cardinal sign of ocular inflammation, a very common presentation, and one of the most frequent ophthalmologic conditions in primary care. As a nonspecific sign of inflammation, its etiology is highly varied and can range from disorders that do not threaten vision (such as some infectious conjunctivitis, allergic and irritant conjunctivitis, and blepharitis, etc.) to diseases that are potentially harmful to the organ and its function (such as uveitis, acute angle-closure glaucoma, endophthalmitis, etc.). [1]

Through the patient's medical history and examination, the etiology of the red eye can be diagnosed and the treatment to be followed determined accordingly. [1] [2]

In self-limiting conditions, such as viral and irritant conjunctivitis, management is based on symptomatic measures focused on reducing conjunctival hyperemia and itching, relieving the patient's discomfort during the illness. This treatment may include cold compresses, decongestants, and lubricants. In some conditions, such as allergic and bacterial conjunctivitis, in addition to etiological treatment with antihistamines or antibiotics, respectively, symptomatic support measures are also recommended. [1] [2] [3] [4]

Synthetic adrenergic agonists such as phenylephrine, naphazoline, oxymetazoline, and tetrahydrozoline are available as ocular decongestants. After topical application, they produce vasoconstriction at concentrations that do not cause mydriasis. These agents provide symptomatic therapy, as they have no effect on the conjunctival response to antigen. [5]

Red eye is a common problem in the general population, accounting for approximately 15% of ophthalmology consultations and almost 6% of general practitioner visits. Symptoms and signs that may accompany red eye include irritation, itching, discharge, tearing, and eye pain. [6]

The first thing to evaluate in patients with red eye is whether it is caused by a serious eye disease. To rule out a serious ophthalmologic disorder, it is important to inquire about the patient's absence of significant eye pain, blurred vision, and photophobia. [7]

Ocular biomicroscopy is a useful tool that can diagnose the causes of more than 90% of conditions associated with red eye. During the examination, it is important to take an adequate history, detailing the onset of the red eye, the duration of its onset, whether the condition is unilateral or bilateral, associated symptoms, ocular and systemic diseases, use of topical and systemic medications, history of trauma, and contact lens use. [8] [9]

The examination should include a visual inspection of the periocular region, adnexa, ocular surface, and anterior chamber. This allows for differentiation of hyperemia in conditions related to eyelid

abnormalities, peripheral (predominantly in the fornix), perikeratic (greater intensity in the scleral-corneal limbus) , and localized conditions. Alterations in corneal transparency or elements in the anterior chamber, such as hyphema or hypopyon, should be sought. Fluorescein staining can identify de-epithelialized regions. [9]Table 2 summarizes the main causes of red eye, according to the predominant location of the conjunctival hyperemia.

Table ii. Main causes of red eye

Red eye of palpebral or periocular origin	Diffuse superficial red eye
• **Blepharitis** • **Chalazion** • **Stye** • **Orbital cellulitis** • **Dacryocystitis**	• Infectious conjunctivitis • Allergic conjunctivitis • Dry eye syndrome
Perikeratic red eye of intraocular origin	**Localized red eye**
• **Keratitis, corneal erosion** • **Uveitis** • **Glaucoma**	• Subconjunctival hemorrhage • Pterygium • Episcleritis • Scleritis

2. 1.2 Pathophysiology of red eye

Red eye results from vasodilation of conjunctival blood vessels. Vascular tone in mammals is regulated by the activity of adrenergic receptors found in vascular smooth muscle, vascular endothelium, and nerve cell terminals. The contractile activity of smooth muscle cells alters blood vessel diameter. Stimulation of α1 and α2 adrenergic receptors causes vasoconstriction of vascular smooth muscle; meanwhile, stimulation of β adrenergic receptors causes vasodilation. Additionally, stimulation of α1 , α2 , and/or β adrenergic receptors on vascular endothelium will cause vasodilation, possibly by increasing nitric oxide. Basal vascular tone is governed by norepinephrine-mediated vasoconstrictor and vasodilator activity, and red eye occurs when vasodilation predominates. [10]

By analogy, the vasoactive effect of active ingredients with adrenergic agonist activity will depend on the balance between vasoconstrictor and vasodilator activity. Anatomical differences in the

distribution of α1 and α2 adrenergic receptors are important , since $_{\alpha 1}$ receptors are found in veins and arteries, while α2 receptors predominate in veins. Consequently, the effects of the active ingredients will depend on the receptors they activate. [10]

2.1.3 Ocular vasoconstrictors

Ocular vasoconstrictors available over-the-counter in the United States are all α adrenergic receptor agonists but vary in their affinity for the receptors. First-generation vasoconstrictors include phenylephrine, tetrahydrozoline, naphazoline, and oxymetazoline. Phenylephrine, a sympathomimetic amine with selective affinity for the α 1 receptor, and oxymetazoline, an imidazole derivative with approximately 5:1 affinity for α 2: α 1 receptors , are no longer commercially available in the United States. Tetrahydrozoline, an imidazole derivative available since the 1950s, is a selective α 1 receptor agonist Naphazoline, another imidazole derivative, is an agonist with activity at alpha 1 and alpha 2 receptors, approved since 1974 as an ocular decongestant, and available over-the-counter in concentrations of 0.012-0.03% for the relief of ocular redness.[10]

Brimonidine is an adrenergic agonist with a marked affinity for the α 2 receptor compared to the α 1 receptor (1000:1). It was initially approved as an ocular hypotensive in 1996 at a concentration of 0.2%; however, its off-label use in LASIK and pterygium surgery has demonstrated that brimonidine has effects in reducing subconjunctival hemorrhage and conjunctival hyperemia, which led to the development of a 0.025% solution to reduce ocular redness caused by minor irritations. [10]

2.1.4 Mechanism of action of vasoconstrictors

α1, mixed α1 and adrenergic agonists α 2, and α 2 selective vasoconstrictors produce constriction of conjunctival blood vessels through the activation of adrenergic receptors, this activation initiates a signal cascade of the G protein that culminates in the contraction of vascular smooth muscle. Specifically, the activation of the α 1 receptor, linked to the Gq protein, produces the contraction of vascular smooth muscle through the inositol triphosphate (IP_3) pathway, while the activation of the $\alpha_{2\ receptor}$, linked to the Gi protein, produces the contraction of the smooth muscle by the intracellular decrease of cyclic adenosine monophosphate (cAMP). Due to the differences in the distribution of α_1 and α 2 receptors , vasoconstrictors with activity in the α 1 receptor produce vasoconstriction of the conjunctival venules and arterioles; while those selective to the α 2 receptor act only on the venules.[10]

2.2 Background on the investigational product

The combination of naphazoline with hypromellose and menthol in ophthalmic solution is indicated to relieve eye redness caused by minor eye irritations. [11] [12] [13]

These medications can reduce vascular congestion of the corneal and conjunctival surface in various disorders affecting the eyeball and/or ocular adnexa. [11] [12] [13]

PRO-185 ophthalmic solution is a solution containing 0.03% naphazoline and 0.2% hypromellose. It also contains menthol among its excipients, distinguishing it from other products that are a combination of naphazoline and hypromellose.

2.2.1 Naphazoline

Naphazoline, classified by its chemical structure as an imidazole derivative, differs from other adrenergic agonists by the replacement of the benzene ring with an unsaturated ring. In general, naphazoline has a higher affinity for the α-adrenergic receptor than for the β-adrenergic receptor. After application to the ocular surface, naphazoline induces marked vasoconstriction by stimulating α-adrenergic receptors on vascular smooth muscle. Imidazole derivatives, such as naphazoline, have the advantage over phenylephrine in that they are less likely to induce rebound congestion and mydriasis. [12] [14] [5]

In most cases of conjunctival hyperemia, naphazoline has an immediate and prolonged vasoconstrictive effect on the bulbar and eyelid capillaries of the conjunctiva. If patients have superficial corneal vessels, they may also be constricted. [14]

Naphazoline can lower intraocular pressure by agonizing α-2 adrenergic receptors, thereby decreasing aqueous humor formation. The pressure reduction effect of naphazoline depends on the dose administered and the patient's sensitivity. [15]

Systemic absorption has been reported after topical application of naphazoline solutions. Although not administered systemically, it can be absorbed through the gastrointestinal tract. The onset of naphazoline's action (conjunctival vasoconstriction) can be felt within 10 minutes and last up to 6 hours. [16]

Hurwitz and Thompson studied the effects of 0.1% naphazoline in over 100 subjects with congested and normal eyes. Slit-lamp evaluations revealed constriction of the conjunctival vessels, with no effects on the deep vessels. No accommodative effects were observed at this concentration. [5]

The incidence of serious adverse reactions is low in patients receiving naphazoline ophthalmic solution at therapeutic doses. Excessive doses and/or prolonged use may irritate the conjunctiva and cause systemic adverse effects. [17]

Ophthalmic naphazoline may cause blurred vision, mild transient itching, irritation, mydriasis, and increased or decreased intraocular pressure. Application of naphazoline to the conjunctiva may release pigment granules from the iris. Rebound congestion, characterized by reactive hyperemia, frequently occurs with prolonged use. [17]

Systemic effects it can occasionally cause include headache, hypertension, cardiac irregularities, nervousness, nausea, dizziness, weakness, and sweating. Overdose can cause drowsiness and decreased body temperature, bradycardia, hypotension, and coma. [17] [18]

Naphazoline poisoning is uncommon, although it can theoretically result in significant morbidity, especially in children, although it is very rare to see more than minimal effects. The toxic dose for

oral ingestion is not established, but children have developed severe reactions after ingesting 1 or 2 milliliters of ocular or nasal formulations containing imidazole derivative decongestants. [18]

2.2.2 Hypromellose

Hypromellose is a white, yellowish-white, or grayish-white powder that dissolves in water to form colloidal solutions. It is used in the pharmaceutical industry to make a film coating for tablets, as a tablet binder, emulsifier, matrix modifier, suspending agent, and stabilizer for gels and ointments. In the food industry, it is used as an emulsifier and stabilizer. In ophthalmic solutions, it is used to prolong the action of some medications (as an excipient) or as an ocular lubricant. It has also been used intraocularly in concentrations of 2% as a medical device for eye surgery. [16]

Hypromellose is a chemically inert substance with a refractive index similar to that of the cornea, and has emollient and cohesive properties. It blends well with other polymers and substances present in artificial tears. It improves viscosity and prolongs the corneal retention time of solutions. [5]

Hypromellose does not have the capacity for intraocular penetration or systemic absorption, and its pharmacokinetic properties have not been studied. [19]

In recent years, ether-type cellulose substitutes, particularly hydroxymethylcellulose and hydroxypropylmethylcellulose, have been used more frequently. They are somewhat less viscous than methylcellulose, but possess cohesive and emollient properties equal to or superior to those of methylcellulose. Like methylcellulose, these ethers mix well with other polymers and substances present in artificial tears, and are compatible with many active ingredients and chemicals used in the eyes. Toda et al. found that 0.5% hydroxypropylmethylcellulose solution improved ocular symptoms in patients with dry eye associated with Sjögren's syndrome, and in those without it as well, although tear break-up time and rose bengal and fluorescein staining improved significantly only in the Sjögren's group. [5]

Ocular surface damage in patients with Sjögren's syndrome is severe due to poor retention of tear components . Tear substitutes are ineffective due to their short retention time on the eye. However, hypromellose 0.5% was found to increase the tear evaporation rate at 40% ambient humidity (TEROS 40) for a longer period than sodium hyaluronate 0.1% or saline-based artificial tears. Hypromellose remains on the ocular surface longer and maintains its moisture. [20]

Cellulose ethers, such as hypromellose, can absorb the tear film layer at the corneal-tear interface, stabilizing a thicker layer of fluid adjacent to the absorption site. The observation that these compounds prolong tear break-up time supports this assumption. [5]

Hypromellose is a substance permitted for oral intake in humans, with an approved dose of 5 mg/kg/day, and no effects have been demonstrated that influence carcinogenesis, teratogenesis, and alterations in fertility with its ingestion. [21]

2.2.3 Menthol

Menthol is a cyclic alcohol found as the main compound in the essential oils of *Mentha canadensis* and *Mentha X piperita.* Menthol is known for its cooling properties when inhaled, consumed, or applied to the skin, due to its ability to chemically activate the cold-sensitive cation channel, also known as transient receptor potential melastin-8 (TRPM8). Studies have shown that menthol acts through the TRPM8 receptor by increasing the rate of intracellular calcium and mobilizing flow through the channels to induce a cold-like response at the site of application. Studies have shown that the cooling effect of menthol can last up to 70 minutes or more in 65% of humans. [22]

Menthol has been used medicinally since ancient times and is frequently used in topical preparations for analgesic, antipruritic, and anti-inflammatory properties, as well as to enhance the penetration of other active ingredients. Menthol has been used in ophthalmic preparations to relieve visual fatigue and discomfort.[23]

The cornea is innervated by three types of afferent neurons with free terminal endings. Mechanoreceptive neurons respond exclusively to mechanical stimuli from the corneal surface; polymodal neurons respond to mechanical, thermal, and chemical stimuli (including pH decreases); and cold-responsive neurons. Activation of mechanoreceptive and polymodal receptors evokes irritation, pain, and a tearing reflex; conversely, activation of cold-responsive neurons induces tearing without irritation or pain. [23]

In addition to low temperatures, the activity of cold-sensitive cells is increased by the application of menthol, a TRPM8 receptor agonist, and by the use of hypertonic artificial tears. [23]Menthol application to the eye can increase tear production. [24]

The irritancy and toxicity of menthol was evaluated by the Draize test in 30 rabbits divided into 5 groups, each of which received solutions with concentrations of 0, 0.025, 0.05, 0.1, and 0.2% in a phosphate buffer at pH 7.4. Each application used 100 µl of the solution, and the dosage was 4 times a day for 7 days. The condition of the ocular tissue was observed at 1, 12, 24, 48, and 72 h after the last application. Corneal opacity and iris hyperemia were graded on a scale of 0-4, while conjunctival congestion, edema, and secretion were evaluated on a scale of 0-3, 0-4, and 0-3, respectively. For each solution, the average values of 4 eyes were calculated. According to the way the tests were evaluated, the concentrations were considered to be non-irritating at average values of 0-3.9, slightly irritating for values of 4-8.9, moderately irritating for values of 9-12.9 and seriously irritating for values of 13-16. [25]

The mean values of the menthol irritation test were 0.0, 0.5 ± 0.5, 1.2 ± 0.8, and 4.2 ± 1 for the 0, 0.025, 0.05, 0.1, and 0.2% solutions, respectively. The results showed that menthol was not irritating at concentrations ranging from 0.025% to 0.1%, and caused mild irritation at the 0.2% concentration. In this study, no visible damage was observed, nor were any abnormal clinical signs reported in the cornea, iris, or conjunctiva with the administration of any of the concentrations. All signs of mild irritation diminished and disappeared completely 48 h after the last application. [25]

Menthol is an excipient in the PRO-185 solution. It is present at a concentration 80 times lower than the concentration that demonstrated mild irritation in the repeated-dose irritability test, and 10 times lower than the lowest concentration that demonstrated no irritating effects. Therefore, no additional safety data are expected from its inclusion as an excipient in the PRO-185 formulation. [25]

2.3 Background on the investigational product

We do not have clinical studies of an ophthalmic solution containing naphazoline, hypromellose, and menthol. However, we have experience in clinical studies with naphazoline and hypromellose at higher concentrations than PRO-185, as well as in a preclinical safety and toxicity study of PRO-185 ophthalmic solution.

2.3.1 Preclinical safety and toxicity study of PRO-185 solution

To determine the safety of PRO-185 (an ophthalmic solution containing naphazoline, hypromellose, and menthol), a preclinical study was conducted to compare its non-inferiority in terms of safety and toxicity with that of a currently marketed naphazoline ophthalmic solution (Nazil® Contamination). In the study, the products were administered to the ocular surface of New Zealand albino rabbits (n=16 subjects). One drop was administered four times daily to the right eye for 30 days, while the left eye served as the control eye. [26]

In the study, 100% of all subjects in both groups and their respective control eyes presented corneal and conjunctival fluorescein staining between grades 0 and 2, values considered normal. At visits 2, 3 and 5, 100% of cases in the PRO-185 and Nazil ® Contamination groups presented grade 0 (absent) fluorescein staining. Only at visit 1, two cases in each group presented grade 1 (mild), this finding not being statistically significant (E. Fisher, p=1.000). [26]

Similarly, for conjunctival fluorescein staining, at visits 2, 3, 4, and 5, 100% of subjects in both groups had grade 0 (absent). While at visit 1, one case in the PRO-185 group was grade 2 (moderate) and one case in the Nazil ® Contamination group was mild, these findings not being statistically significant (X^2 , p = 0.368). In the subjects in the PRO-185 group, only at visit 1, two cases for the treated eyes presented grade 1 (mild) corneal staining with fluorescein, not being statistically significant with respect to the control eyes (E. Fisher, p = 1.000). For conjunctival fluorescein staining, at visit 1, both one treated eye and one control eye presented grade 1 staining (E. Fisher, p = 1.000). [26]

At the initial, 3, 4 and 5 visits, 100% of the treated eyes in both groups showed grade 0 (normal) conjunctival hyperemia. At visit 1, two cases were mild and one was moderate in the PRO-185 group, compared to 3 mild cases in the Nazil ® Contamination group, although these findings were not statistically significant (X^2 , p=0.549). However, in this case, one eye in the PRO-185 group presented grade 2 conjunctival hyperemia, compared to none in the Nazil ® Contamination group. At visit 2, only one case in the Nazil ® Contamination group presented mild hyperemia (E. Fisher, p=1.000). [26]

In subjects treated with PRO-185, only at visit 1, two cases for the treated eyes presented grade 1 (mild) conjunctival hyperemia and one case grade 2 (moderate), while in the control eyes only mild

conjunctival hyperemia was reported in one eye. This difference was not statistically significant (X^2, p=0.435). [26]

In the eyes treated with Nazil® Contamination, at visit 1, three treated eyes, compared to one control eye, had mild hyperemia (E. Fisher, p=0.569). At visit 2, both one treated and one control eye had mild hyperemia (E. Fisher, p=1.000). [26]

Only one case at visit 1 in the PRO-185-treated group showed minimal conjunctival edema; this finding was not statistically significant between treatments (E. Fisher, p=1.000). All treated and control eyes had normal conjunctival discharge during the study, as well as normal corneal opacity. [26]

At the initial, 2, 3, 4 and 5 visits, 100% of the treated eyes in both groups presented normal tearing. At visit 1, five eyes in the PRO-185 group and five in the Nazil ® Contamination group presented grade 1 tearing (increased tear level with concave meniscus), while one eye in each group presented grade 2 tearing (increased tear meniscus level with convex meniscus). These findings were not statistically significant (X^2, p = 1.000). [26]

In subjects treated with PRO-185, only in visit 1, five cases for the treated eyes (OD) presented grade 1 and one case grade 2, compared to four control eyes grade 1 tearing, without these findings being statistically significant (X^2, p=0.411). [26]

For subjects treated with Nazil ® Contamination, only in visit 1, five cases for the treated eyes (OD) presented grade 1 and one case grade 2, compared to three control eyes grade 1 tearing, without these findings being statistically significant (X^2, p=0.248). [26]

No statistically significant differences were observed between groups in intraocular pressure during the execution of the study. The initial mean ± standard deviation (SD) value for PRO-185 was 8.88 ± 0.64 mmHg vs 9.13 ± 1.55 mmHg for Nazil ® Contamination (Mann-Whitney, p = 0.824). While the final mean ± SD IOP was 9.38 ± 1.06 mmHg for PRO-185 vs 8.75 ± 1.28 mmHg for Nazil ® Contamination (Mann-Whitney, p = 0.354). Chart 1shows the IOP during the execution of the protocol. [26]

No statistically significant differences were observed between treatment groups for the mean change in IOP during the execution of the protocol (Mann-Whitney, p>0.05). Chart 2shows the variation in IOP for both treatments. Only one subject (subject 12) treated with PRO-185 showed an increase of 5 mmHg at visit 2 compared to visit 1 (IOP visit 2 of 11 mmHg and IOP visit 1 of 0.6 mmHg). Despite the significant difference between the intraocular pressure at visits 2 and 1 of subject 12, if the intraocular pressure at visit 2 is compared with the baseline visit, the difference is 3 mmHg (baseline visit IOP of 9 mmHg and visit 2 IOP of 11 mmHg), this difference being insufficient to declare treatment-induced ocular hypertension in the study subject. [26]

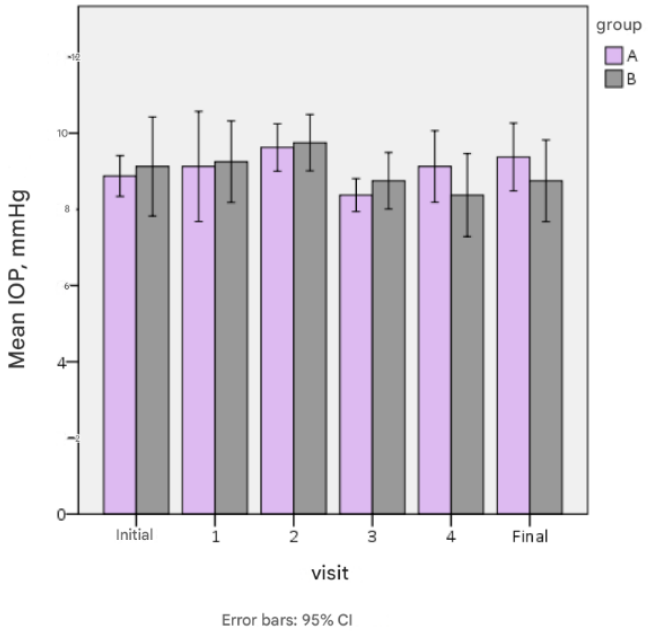


Chart 1Intraocular pressure (IOP) in mmHg during the study. Group A = PRO-185 Group B = Nazil® Contamination

In Chart 1, the bars show the mean value ± 95% CI for the error bars. Mann-Whitney U p>0.05 in all comparisons.

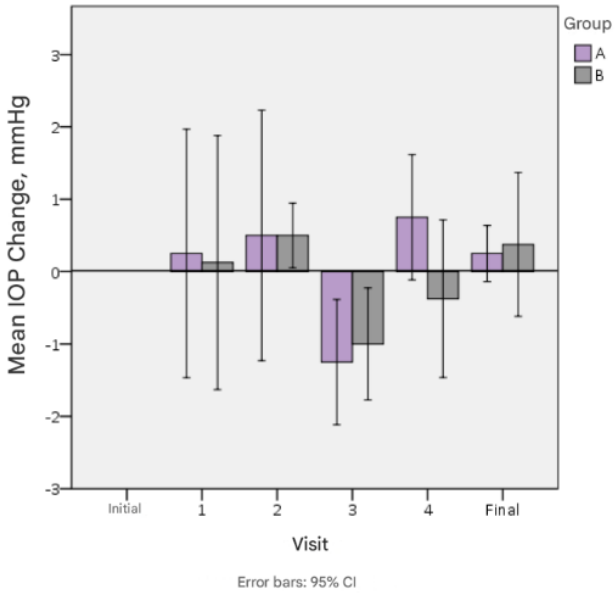


Chart 2Change in intraocular pressure (IOP) in mmHg during the study. Group A = PRO-185 Group B = Nazil® Contamination

In the Chart 2**, the** bars show the mean value ± 95% CI for the error bars. Mann-Whitney U p>0.05 in all comparisons. [26]

For eyes treated with PRO-185, compared to their control eyes, no statistically significant differences in IOP were observed during the execution of the protocol (Mann-Whitney, p>0.05), see Chart 3The IOP at visit 3 (8.38 ± 0.52 mmHg) was statistically lower than the IOP at visit 2 (9.63 ± 0.74 mmHg), for eyes treated with PRO-185 (Wilcoxon, p=0.026). [26]

In eyes treated with Nazil ® Contamination, compared to their control eyes, no statistically significant differences in IOP were observed during the execution of the protocol (Mann-Whitney, p>0.05), see Chart 3The IOP at visit 2 (9.75 ± 0.87 mmHg) was statistically higher than the IOP at visit 1 (9.25 ± 1.28 mmHg), (Wilcoxon, p=0.046). Meanwhile, the IOP at visit 3 (8.75 ± 0.89 mmHg) was statistically lower than its mean value at visit 2 (Wilcoxon, p=0.038). [26]

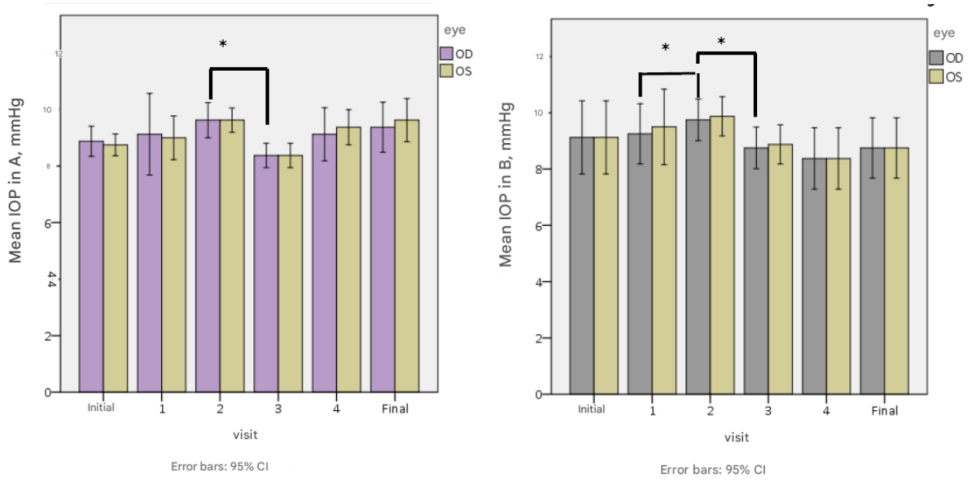


Chart 3Intraocular pressure (IOP) in mmHg for eyes treated with PRO-185 (OD) (Group A) relative to their control eyes (OS) and for eyes treated with Nazil® Contamination (OD) (Group B) relative to their control eyes (OS)

In Figure 3, the Wilcoxon signed-rank test for related samples was performed in PRO-185, for visit 2 > visit 1 and in Nazil ® Contamination, for visit 2 > initial visit and visit 2 > visit 3; *p<0.05. [26]

No adverse events occurred for either treatment during the protocol. Similarly, no alterations of any kind were observed for either treatment arm during the protocol. [26]

In the histopathological results: there were no changes in the cornea, conjunctiva, ciliary body, retina, optic nerve for either treatment group. Regarding the goblet cells, the Subjects assigned to PRO-185 treatment had a mean ± SD value of 21.50 ± 2.33 cells (AAPas , %) compared to 22.25 ± 2.71 cells (AAPas , %) in the Nazil® Contamination group , which was not statistically significant (Mann-Whitney, p = 0.546). Cell density in both the PRO-185 and Nazil ® Contamination treatments was not different in treated eyes compared to their controls (p values = 0.788 and 0.580 respectively). [26]

In general, the results of the variables studied do not show significant differences between the two products, nor do they show any unexpected abnormal results that could indicate the existence of any risk of these products in the research subjects. [26]

Therefore, since there was no difference greater than 10% between the incidence of the main variables, we can accept the alternative hypothesis of the study, which states: The safety of the PRO-185 solution is not inferior to the safety of the Nazil ® Contamination solution within a non-inferiority margin of 10% in abnormal fluorescein uptake and/or in the incidence of conjunctival hyperemia grade ≥ 2; given that there were no significant abnormal values in the secondary and toxicity variables with the administration of the product, and since it has been established by the main variables that the PRO-185 solution is not inferior in safety to Nazil ® Contamination, we can be certain that the product was safe to use with the animal model of the study (New Zealand albino rabbits). [26]

2.3.2 Preclinical study of an ophthalmic solution of naphazoline 0.1% and hypromellose 0.5% (higher concentration of the main components of PRO-185)

Ten healthy New Zealand albino rabbits were administered a 0.1% naphazoline and 0.2% hypromellose solution four times daily for 10 days as part of a preclinical study to evaluate the product's safety and acute toxicity. The study results showed no changes to the ocular surface of the rabbits receiving the drug; only two rabbits exhibited minimal ocular secretion on the first day of the study. No dye uptake was observed in any of the rabbits. The study concluded that the 0.1% naphazoline and 0.2% hypromellose ophthalmic solution is safe for topical application to the ocular surface of healthy albino rabbits. [27]

In another preclinical study, the safety and acute toxicity of naphazoline administered to the ocular surface of healthy New Zealand rabbits was evaluated. Two groups were evaluated with naphazoline; one group received Nazil Ofteno®, the other Naphacel Ofteno® (naphazoline 0.1% and hypromellose 0.5%), and a third group received placebo. Each group consisted of 14 rabbits. The rabbits received the study product 4 times daily for a period of 30 days. [26]

On days 15 and 30 of the study, the Nazil Ofteno® group presented conjunctival hyperemia, 72% of the eyes evaluated, while 68% of those treated with Naphacel Ofteno® had hyperemia, and 54% of those treated with placebo. [26]

Comparing the intensity between both naphazoline groups, on day 5 the hyperemia was more intense for Naphacel Ofteno®, and on day 15 for Nazil Ofteno®. [26]

There was no conjunctival discharge during the study in any of the groups, nor any changes in the posterior segment. There was also no corneal staining with fluorescein, lissamine green, or changes in intraocular pressure in the rabbits in all three groups. [26]

This study concluded that Nazil Ofteno® and Naphacel Ofteno® have a similar safety and toxicity profile. [26]

2.3.3 Clinical study of an ophthalmic solution of naphazoline 0.1% and hypromellose 0.5%

An open-label, single-center, observational, prospective clinical study included thirty healthy volunteers. Volunteers were administered a 0.1% naphazoline and 0.5% hypromellose solution four times daily for ten days. Results showed no changes in conjunctival hyperemia, conjunctival discharge, ciliary injection, chemosis, and rose bengal and fluorescein staining. However, 86% of patients experienced burning, 70% of which was mild and the remainder moderate; 6.6% had mild red eyes; and 6.6% had mild ocular pain. The mean burning score was 0.6861, with a standard deviation of 0.42 and a standard error of 0.016. Student's t-test showed a $p>0.05$, leading to the conclusion that the burning score was not statistically significant. The study concludes that the solution of naphazoline 0.1% and hypromellose 0.5% is safe for application to the ocular surface of healthy volunteers. [28]

2.4 Background on the research

2.4.1 About the research question

Is the application of PRO-185 ophthalmic solution safe and tolerable in clinically healthy subjects?

The importance of the question preceding this paragraph is that there are no clinical studies with products identical to the PRO-185 solution, although there are studies with similar products (different concentration and excipients).

2.5 Risk-benefit evaluation

2.5.1 Known potential risks

Formulations containing naphazoline and/or hypromellose at the same concentrations as PRO-185 are available over-the-counter without the need for clinical or preclinical studies in high surveillance countries.[11] The risks associated with the use of this product in specific populations are described below; however, the use of this medication in the study will be in a population group without the alterations or characteristics described below.

Patients with high blood pressure, cardiovascular abnormalities, hyperglycemia, hyperthyroidism, infections, and corneal wounds should use products containing naphazoline with caution due to the risk of aggravation of these conditions.[29]

Patients with angle-closure glaucoma or closed angles may experience significant increases in intraocular pressure and should not use products similar to PRO-185.[29] [30] [31]

Overuse of this product may cause rebound conjunctival hyperemia.[29] [11]

Some patients, when using this product as recommended, may experience pharmacological mydriasis, with the resulting decreased vision.[11]

Ophthalmic vasoconstrictors, such as this product, should not be used in children under 12 years of age, and serious reactions requiring hospitalization have been reported with accidental ingestion of naphazoline products by children.[29] [32]

Administration of this product during pregnancy and lactation is prohibited.[29] [32]

Although menthol is present as one of the components, it is an excipient and is present at a concentration 80 times lower than the concentration that demonstrated mild irritation in repeated-dose irritability tests, and 10 times lower than the lowest concentration that demonstrated no irritating effects. Therefore, no additional safety data are expected from incorporating it as an excipient in the PRO-185 formulation. Furthermore, there is already experience in other markets with the use of eye drops with menthol as an excipient, and a lubricant and vasoconstrictor.[25]

2.5.2 Known potential benefits

The benefit of the drug in the target population is a reduction in hyperemia or ocular redness. [11] [12] [13]However, as this is a Phase I study, the study population will be a healthy population. Therefore, no direct benefit is expected for each individual, and the benefit of the research will be to understand the formulation's behavior and to have more data on its safety in order to prevent or identify adverse effects in users or problems with tolerance.

2.6 Problem statement

There are many formulations on the market containing naphazoline or hypromellose, some of them a combination of both. However, although there is experience with these products in the post-marketing stages, there are no published clinical studies that have examined the behavior of these drugs.

Since these products can be approved for marketing directly, without the need for clinical trials, there is little information available in the medical literature on these products. Furthermore, when they are marketed as over-the-counter products, the information included in the instructions or prescribing information is summarized and, in most cases, lacks a description of the experience of the drug's post-marketing behavior.

2.7 Justification of the study

Laboratorios Sophia, SA de CV, has conducted a preclinical and phase I clinical study of a similar formulation, but with a higher concentration of components. The reduction in the concentration of the main components (naphazoline and hypromellose) in the PRO-185 solution compared to this formulation studied could mean greater safety of PRO-185, but not necessarily greater tolerance, since the excipients may be involved in clinical manifestations unrelated to the main components.

Menthol has been used in ophthalmic preparations to relieve visual fatigue and ocular discomfort. [23]The inclusion of menthol among the excipients in PRO-185, as well as the lack of clinical evaluation of this formulation, necessitates clinical evaluation of the product. This would provide a more complete safety and tolerability profile.

In a preclinical safety and toxicity study of PRO-185 ophthalmic solution in New Zealand albino rabbits, the drug was shown to be non-toxic and safe to administer. Information also indicates that, at the menthol concentration used in Draize tests, there were no irritating or toxic effects.

Because there is preclinical evidence of safety and toxicity with the PRO-185 formulation, as well as the absence of irritation at the concentration used; and because there are ophthalmic products with menthol as an excipient in similar combinations on other markets, it was decided to advance to clinical research to test the safety and tolerability of PRO-185 in a controlled environment with healthy subjects.

3. Objectives and hypotheses

3.1 Objectives

3.1.1 Main objective:

- To evaluate the safety and tolerability of PRO-185 ophthalmic solution in ophthalmologically healthy subjects.

3.1.2 Specific objectives:

- To evaluate the safety of PRO-185 ophthalmic solution by measuring the incidence of subjects who present an increase of > 5 mmHg in intraocular pressure after 20 minutes of application compared to the initial value.
- To evaluate the safety of PRO-185 ophthalmic solution by measuring the incidence of subjects experiencing a heart rate variation of >15 beats per minute 20 minutes after application.
- To evaluate the safety of PRO-185 ophthalmic solution by measuring the incidence of subjects with an increase in systolic blood pressure of >15 mmHg or >10 mmHg in diastolic blood pressure 20 minutes after application.
- To assess tolerability by the incidence of grade 3 and 4 conjunctival hyperemia.
- To assess tolerability by the incidence of pharmacological mydriasis.
- Evaluate tolerability by the incidence of expected and unexpected adverse events.

3.2 Hypothesis

H $_0$ = PRO-185 ophthalmic solution is safe and tolerable for ophthalmic use, as it occurred in 20% or less of subjects with any of the following adverse events: increased intraocular pressure (IOP >5 mmHg), heart rate variation (>15 bpm), increased systemic blood pressure [>15 mmHg systolic (SBP) or >10 mmHg diastolic (DBP)], pharmacological mydriasis, or grade 3 and 4 conjunctival hyperemia.

$$H_0: p - p_0 \leq \delta$$

H $_1$ = PRO-185 ophthalmic solution is not safe or tolerable for ophthalmic application because more than 20% of subjects experienced any of the following adverse events: increased IOP (>5 mmHg), heart rate variation (>15 bpm), increased systemic blood pressure [>15 mmHg systolic (SBP) or >10 mmHg diastolic (DBP)], pharmacological mydriasis, or grade 3 and 4 conjunctival hyperemia.

$$H_1: p - p_0 > \delta$$

Comparisons will be made between pre- and post-treatment measurements from visits 1 and 2, and between measurements from the baseline visit (before PT application) and measurements from the

final visit. Only one of the adverse event values described in the hypothesis need be abnormal with a frequency >20% between the comparisons (pre-treatment with post-treatment; or pre-PT baseline with values from the final visit) for the null hypothesis to be rejected.

4. Study design

4.1 General description of the study

Phase I clinical study, controlled, non-comparative, open, single-center

4.2 Justification of the study design

Study design (clinical trial) is considered the highest standard of data quality when exploring the effect of an intervention. The drug development phase (Phase I) corresponds to the study's objective, which is to evaluate safety and tolerability. Therefore, the intervention time is short and the required sample size is smaller than that of an efficacy clinical trial. Blinding was not considered in the study because there will only be one treatment group.

4.3 Expected duration

The total duration of the study, from the first patient visit to the final report, is estimated to be 5 months, with a 4-month recruitment period.

The approximate duration of each subject in the study is 10 days.

5. Study population

5.1 Eligibility criteria

5.1.1 Inclusion criteria

- Be clinically healthy
- Have the ability to voluntarily grant signed informed consent
- Be able and willing to comply with scheduled visits, treatment plan, and other study procedures
- Be between 18 and 45 years old.
- Women of childbearing potential must ensure continued use (started ≥ 30 days prior to signing the ICF) of a hormonal contraceptive method or intrauterine device (IUD) during the study period.
- Have a best-corrected visual acuity of 20/30 or better in both eyes.
- Have vital signs within normal parameters.
- Have an intraocular pressure ≥10 and ≤ 21 mmHg.

5.1.2 Exclusion criteria:

- Be a user of topical ophthalmic products of any kind.
- Being allergic to naphazoline or having a history of intolerance to nasal decongestants or ocular vasoconstrictors.
- Have a history of a suspected diagnosis of primary angle closure, primary angle closure, or angle-closure glaucoma.
- Having iridotomies or waiting for iridotomies.
- Have conjunctival hyperemia grade 3 and 4 on the Efron scale.
- Have ocular surface staining with a value equal to or greater than 3 on the SICCA scale in either eye.
- Being a user of medications or herbal products, by any other route of administration.
- For women: be pregnant, breastfeeding, or planning to become pregnant during the study period.
- Having participated in clinical research studies 90 days prior to inclusion in this study.
- Having previously participated in this same study.
- Be a contact lens user who cannot discontinue use during the study.
- Having a history of any chronic-degenerative disease, including diabetes and high blood pressure.
- Present inflammatory or infectious disease, active at the time of entering the study.
- Present unresolved injuries or traumas at the time of entering the study.
- Have a history of any type of eye surgery
- Having undergone non-ophthalmological surgical procedures in the last 3 months.

5.2 Criteria for elimination and/or substitution of subjects

5.2.1 Elimination criteria

- Withdrawal of informed consent.
- Major deviation from the protocol that could impact the integrity of the results.
- Presentation of an adverse event, whether or not related to the investigational product, which, in the opinion of the PI and/or the sponsor, could affect the subject's ability to safely continue the study procedures.
- Non-tolerability or hypersensitivity to any of the compounds used during the tests.
- Non-tolerability or hypersensitivity to the investigational product.

5.2.2 Subject substitution

The sponsor, with prior authorization from the research ethics committees, may decide to replace subjects who withdraw their FCI or those who are lost to follow-up, if it is necessary to balance the study groups so that they are evaluable.

5.3 Scrutiny failures

A screening failure is defined as a participant who agrees to participate in the study, giving their consent, but who is not assigned to a treatment group; that is, they do not enter the study. The following information regarding screening failures must be reported, at a minimum:

- Demographic data.
- Details of the counting failure (specify whether due to eligibility criteria, which one, or some other reason for the failure).
- Presence of serious adverse events during the scrutiny.

The above is necessary to comply with the CONSORT guidelines (*Consolidated Standards of Reporting Trials*) for the publication of results or to respond to potential questions from regulatory authorities.

5.4 Recruitment and retention strategies

The subject's participation in the study lasts approximately 10 days, during which they will attend four visits in total, corresponding to the baseline, first, second, and final visits. Strategies to improve subject retention include, but are not limited to:

- Clearly report the objectives of the study.
- Make calls or send text messages to remind yourself of appointments or activities to do.
- Provide a printed calendar and ID card to remind you of upcoming appointments and activities, as well as their estimated duration.

- Systematic organization of the study procedures, so that the subject does not stay longer than necessary during his visit.
- Minimize subject wait times.

All materials to be delivered to the subject or recruitment strategies implemented by the center will be submitted for approval by the corresponding committees.

5.5 Procedure in case of early discontinuation

For this protocol, early discontinuation is defined as those subjects who were assigned the PI, who at some point were active subjects in the study, but their final evaluation could not be completed.

If the subject does not complete their participation due to withdrawal of consent or a major deviation, the last visit at which their withdrawal was determined will be considered their final visit. Subjects withdrawn due to the presence of AEs will continue the follow-up as determined until their AE is resolved.

In cases where the participating subject does not attend their appointment, the research site will call to determine the reason and will attempt to schedule a new appointment within the established window or an unscheduled appointment. If an appointment cannot be scheduled, the subject will be considered lost to follow-up, and the presence of adverse events and the reason for discontinuing the study will be asked as minimum data.

5.6 Subject identification

Study subjects will be identified by a number and the initials of their name.

The initials of the subject of study will be obtained starting with the first letter of the name, followed by the first letter of the first surname and the first letter of the second surname, obtaining a maximum of three letters. In case the person has two names or a compound surname, the first letter will always be used.

Example:

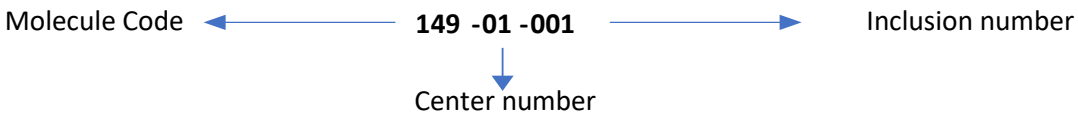
TO. A rieh Daniel M ercado C arrizalez B. J uan D e la Torre O rozco
to. Initials: AMC b. Initials: JDO

During the counting stage, you will be assigned a participant number consecutively, using 3 consecutive digits.

Once the subject has been selected, they will be assigned a number that will identify them throughout the study. This code will consist of eight numbers in the following order from left to right:

- three digits of the molecule under study according to the name given by the sponsor.
- two digits corresponding to the research center number.
- three digits of the consecutive number assigned to its inclusion in the research center.

Example of assigned number:



6. Investigational product and treatment

6.1 Managed Products

6.1.1 Investigational Product

- PRO-185. Naphazoline 0.03% and hypromellose 0.2%. Solución oftálmica. Laboratorios Sophia, S.A. de C. V., Zapopan, Jalisco, Mexico.
- Route of administration: Ophthalmic.

6.1.2 Dose of the investigational product

- Dosage: 1 drop 4 times a day in both eyes (AO).

6.1.3 Treatment with the investigational product

The investigational product will be administered at the conclusion of the baseline visit, and the subject will administer the drops approximately every 2-4 hours between drops, applying them a total of 4 times per day to each eye.

Baseline visits, 1, and 2, should be completed in the morning, before 11 a.m., to allow the patient more time to apply PRO-185 throughout the day. During visits 1 and 2, the patient will come to the clinic without having previously applied the investigational product. The first application will be considered the drop applied by the researcher during the visit. For visit 1, the patient will be required to apply the product three times after the visit has concluded. For visit 2, the patient will not need to apply the product again because the investigational product will have been returned to the researcher.

6.2 Storage and handling of the investigational product at the study center

Delivery will be made via a courier service contracted by the sponsor, specifically selected for this purpose, to the address of the research center in accordance with the study plan.

Reception will be carried out by the assigned research team staff. They must verify the condition of the primary packaging (box). If it shows alterations or defects in its integrity that, in their judgment, could have damaged the contents, they must report this to the sponsor. If the package shows no significant defects, they will proceed to open it.

Inside the shipment, you must locate the receipt and temperature *data logger* . You must verify that the recorded temperature meets the specifications for its transport and storage. You will verify the contents (PI) with what is reported on the document. If the document matches the contents, you will sign the receipt and send it to the sponsor. If not, you will notify the sponsor.

Storage and safeguarding are the responsibility of the research center. The medication must be kept in a secure area with restricted access.

Storage temperature should be 2° to 30°C.

Upon receipt at the center and until the PI is out of stock, the research center is required to review the PI storage conditions daily and manually record the temperature recorded by the *data logger* (current, minimum, and maximum temperatures) in the designated format . These data will be reviewed by the clinical monitor during their monitoring visits, based on the records stored in the data logger's memory .

In the event of material loss, this must be documented in the input and output log along with a clear description of the mechanism by which the loss occurred.

Upon completion of the protocol, all study materials will be retrieved by the sponsor as part of the closing visit. The final return of materials will be made by the principal investigator or the person designated by the principal investigator to return materials at the end of the study.

The sponsor reserves the right to initiate civil and criminal action against the principal investigator in the event of undocumented material missing at the end of the study.

6.3 Concomitant treatments and medications (permitted and prohibited)

Any medication used, in addition to appearing in the clinical note, must be recorded in the concomitant medications section of the eCRF .

Permitted medications:

- Ophthalmic:

All permitted medications administered ophthally during the study must wait a minimum of 10 minutes after the last application of the study or reference treatments. This is to avoid treatment interactions with the tear film, based on the physiological tear flow rate and volume. [24]

    - Tetracaine 0.5%
    - Tropicamide 0.8% / Phenylephrine 5%
    - Hypromellose 2%
    - Fluorescein
    - Lissamine green
- Systemic or other routes of administration other than ophthalmic:
    - Any hormonal contraceptive will be included, including tablets, injections, and drug-releasing devices.

Prohibited medications:

- Any medication with ophthalmic application that is not on the list of permitted medications
- Any systemic medication that is not on the list of permitted medications.

6.4 Procedure for monitoring and measuring adherence

For over four decades, numerous investigations have been conducted on the appropriate way to measure and quantify medication adherence, however, none has reached a consensus to establish itself as the gold standard, both in cross-sectional and longitudinal studies. [25, 26, 27, 28, 29, 30, 31, 32]

There are different procedures for measuring adherence to pharmacological interventions. The most common procedure involves self-reports, which include patient interviews, questionnaires, and self-monitoring diaries. Their strengths are speed, flexibility, low cost, and ease of implementation; they have a high degree of specificity for nonadherence; however, their sensitivity and reliability for adherence are low. [32, 33]

Biochemical measurement of the drug, or its metabolite, is one of the methods that best confirms drug use. However, in addition to being costly and impractical, it is of little use in ophthalmic applications, as peripheral concentrations may be undetectable; and samples from other tissues require more invasive methods that would not be advisable. [32]

Medication counting is another way to measure adherence. Classically referred to as "pill counting," in ophthalmology it is translated as the weight of the bottle. This is a simple, inexpensive, and noninvasive method. The main disadvantages of this method are: 1. It cannot confirm the application of the medication (it could have been intentionally dropped or instilled outside the eye), and 2. It depends on the subject bringing the medication back. [32, 33]

Adhesion assessment will be based on the bottle's weight and will be performed taking into account the following information: drop weight, initial container weight, final container weight, and the total number of applications. The following simplified formula will be used:

$$Ad = \frac{(P_i - P_f)100}{P_T}$$

Where:

Ad = adhesion

P_i = weight of the container delivered to the subject at the beginning

P_f = weight of the container returned by the subject

P_T = weight of the dosage indicated for the investigational products

$$P_T = (P_g)\,G$$

Where:

P_g = weight of one drop of the medicine, determined by the research and development department

G = number of applications indicated for the investigational products

Containers that do not maintain their physical integrity will not be considered for the adherence calculation. Subjects who do not return the investigational product container by Visit 2 will be considered non-compliant.

There is no standardized parameter to define adequate adherence; this must be defined and outlined by the objectives of the particular research. [32]

For this study, a minimum adherence of 80% (by weight and diary) will be considered necessary to meet the research objectives. Therefore, subjects with less than 80% adherence will be included in the intention-to-treat population.

6.5 Strategies to improve adherence

1. The PI will educate the subject on the importance of correctly administering concomitant treatment to achieve the study objectives.
2. Direct questioning by the IP regarding the application of concomitant treatment.
3. Delivery of a printed calendar specifying the date of the visit and its activities.

If deemed necessary, text messages may be sent as reminders. The content of these messages must be approved in advance by the IEC.

7. Methods and procedures of the study

7.1 Research center

This study will be conducted at a research center previously evaluated by the sponsor. The center will be an institution or facility that conducts health research and complies with current regulations.

The research center will be responsible for forming a multidisciplinary research team to execute the clinical study according to the protocol. It is its prerogative to design the organization and select the personnel who will perform these functions. However, the sponsor requires that the PI and sub-investigator be physicians specializing in ophthalmology.

Any person assigned, under the PI's responsibility, to a part of the study monitoring (sub-investigator, nurse, etc.) or a specific role in the study (pharmacist, administrative assistant, study coordinator, etc.) must be listed in the "Delegation of Responsibilities."

The competency and training of all individuals directly involved in study activities must be verified prior to the conduct of any protocol-related activities. This must be recorded, and documents constituting evidence of this competency and/or training must be retained in the study master file. The competency and training of personnel involved in the study, both at the central level and at the study site, are the responsibility of the sponsor.

The sponsor must ensure that all study site personnel participating in the study are adequately trained in the study (research protocol, investigator's manual, amendments, standard operating procedures, etc.) and in ICH Good Clinical Practices prior to the start of their participation in the study. Training must be documented in writing and filed in the study master file.

7.2 Clinical study registration

This clinical study will be registered by the sponsor in public clinical trial registries prior to its initiation (inclusion of the first subject): the National Registry of Clinical Trials (RNEC) of the Federal Commission for the Protection against Sanitary Risks (COFEPRIS) and on a WHO primary registry platform. WHO primary registries meet specific criteria regarding content, quality and validity, accessibility, unique identification, technical capacity, and administration. WHO primary registries meet the requirements of the International Committee of Medical Journal Editors (ICMJE).

7.3 Treatment assignment

- This is an open-label, comparative study. All patients will receive the same treatment.

7.4 Outcome variables

7.4.1 Primary outcome variables

– Intraocular pressure.

- Heart rate.
- Systemic blood pressure.
- Pupillary diameter.
- Conjunctival hyperemia.

7.4.2 Secondary outcome variables

- Best corrected visual acuity (BCVA).
- Changes in ocular surface staining.
- Incidence of chemosis.
- Incidence of expected adverse events.
- Incidence of unexpected adverse events.

7.4.3 Exploratory variables

- Respiratory rate.
- Body temperature.

7.4.4 Definition of variables, methods and scales for their measurement

Table iii. Operational definition of variables

Variable	Conceptual Definition	Operational Definition	Type of measurement	Reference value	Statistical test
Intraocular Pressure	Tonometry is the objective measurement of IOP, based on the force required to flatten the cornea or the degree of corneal indentation produced by a fixed force.	By means of Goldmann tonometry based on the Imberk -Fick principle.	Discrete quantitative	10 – 21 mmHg	Wilcoxon one-sample signed-rank test
Heart rate	Pulse is a measurement of HR, that is, the number of times the heart beats per minute.	With each heartbeat, the left ventricle contracts and expels blood into the aorta. This forceful expulsion of blood creates a wave that is transmitted to the periphery of the body through the arteries.	Discrete quantitative	60 – 100 bpm	Wilcoxon one-sample signed-rank test

Variable	Conceptual Definition	Operational Definition	Type of measurement	Reference value	Statistical test
		It is measured with an electronic blood pressure monitor or manually.			
Systemic blood pressure	It is the force exerted by blood against the walls of the arteries. SBP is the pressure inside the artery when the heart contracts and pumps blood through the body; while DBP is the pressure inside the artery when the heart is resting and filling with blood.	Measured with a blood pressure monitor and a stethoscope.	Discrete quantitative	SBP: 120 – 139 mmHg DBP: 80 – 89 mmHg	Wilcoxon one-sample signed-rank test
Pupillary diameter	The natural pupil of the human eye is usually approximately circular, for a given subject, the pattern of aberrations, diffraction, depth of field and retinal illumination depend on the pupil diameter, which in turn varies depending on the ambient lighting.	The pupillary diameter results from the balance between the pupillary sphincter muscle and the radial fibers of the iris, which have only autonomic innervation [33].	Continuous quantitative	2 – 6 mm	Wilcoxon one-sample signed-rank test
Conjunctival hyperemia	It is defined as the simplest reaction of the conjunctiva to a stimulus; a red	Direct observation. Classification using	Qualitative ordinal	Degrees: 0= Normal 1= Very mild 2= Mild	*Binomial* X^2

Variable	Conceptual Definition	Operational Definition	Type of measurement	Reference value	Statistical test
	appearance is observed secondary to vasodilation of the vessels of the conjunctiva of variable intensity.	the Efron scale (see appendix 16.1)		3= Moderate 4= Severe	
Changes in the AVMC	Spatial visual acuity is the ability to distinguish separate elements of an object and identify them as a whole. It is quantified as the minimum angle of separation (located at the nodal point of the eye) between two objects that allows them to be perceived as separate objects.	Snellen chart	Continuous quantitative	0.6 to 2.0	Wilcoxon one-sample signed-rank test
Changes in ocular surface staining	Detection of epithelial defects in the cornea and conjunctiva.	Direct observation with a slit lamp will be graded according to the SICCA (see annex 16.2) [34].	Discrete quantitative	0 to 12	Wilcoxon one-sample signed-rank test
Incidence of Chemosis	It is conjunctival edema, the result of an inflammatory reaction. It is classified as present or absent.	The evaluator will use a narrow beam of light at 60° and measure whether the conjunctiva separates by ≥1/3 of the entire eyelid opening or if it extends beyond the gray line.	Nominal qualitative	Present / Absent	Binomial X^2

Variable	Conceptual Definition	Operational Definition	Type of measurement	Reference value	Statistical test
Incidence of AE	Any adverse medical event that occurs in a patient or clinical research subject who has been administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment [35].	All AEs will be reported (according to the protocol) and not only those in which the PI suspects a causal relationship with the treatments.	• Discrete quantitative • Nominal qualitative	• Incidence • Seriousness (gravity) • Causality	Binomial X^2
Respiratory rate	The process involves inspiration and expiration, diffusion of oxygen from the pulmonary alveoli to the blood and carbon dioxide from the blood to the alveoli, and the transport of oxygen to body tissues and organs.	RR is the number of breaths a person takes per minute at rest.	Discrete quantitative	12 – 16 rpm	Wilcoxon one-sample signed-rank test
Body temperature	A clinical parameter that reflects the physiological state of the body. It is the balance between heat production and heat loss.	Degree of heat or cold, expressed in terms of a specific scale, using a thermometer.	Continuous quantitative	36.7 – 37.4°C	Wilcoxon one-sample signed-rank test

Abbreviations: BCVA, best-corrected visual acuity; AE, adverse event; HR, heart rate; RR, respiratory rate; PI, principal investigator; bpm, beats per minute; DBP, diastolic blood pressure; SBP, systolic blood pressure; IOP, intraocular pressure; rpm, breaths per minute; χ^2, Chi-square.

7.4.5 Description of the variables, methods and scales for their measurement

7.4.5.1 Intraocular pressure

Tonometry is the objective measure of IOP, based primarily on the force required to flatten the cornea, or the degree of corneal depression produced by a fixed force. Goldman tonometry is based on the Imbert-Fick principle. [34]

Tonometry will be performed after instillation of topical anesthetic with fluorescein and the use of a cobalt blue filter (after assessing surface staining). Two readings will be taken, and the average will be recorded in the clinical record. The average will be recorded in the eCRF .

Management as AEs: IOP peaks ≥24 mmHg should be reported as AEs, as well as increases in intraocular pressure greater than 5 mmHg between evaluations before and after application of the investigational product (visits 1 and 2).

7.4.5.2 Heart rate

Heart rate is the number of times the heart's ventricles contract per unit of time, usually per minute. [36]Heart rate measurement will be performed by direct auscultation of the chest with a stethoscope. In the case of women, wrist pulse measurement may be permitted. However, the method chosen for the patient will be the same one used at all visits.

For heart rate measurement, the patient must be calm and at rest. They will be asked to rest for at least 20 minutes upon arrival at the clinic before the measurement, and when the measurement is to be performed after medication administration, they must also rest for 20 minutes.

For protocol purposes, rest is defined as a state in which the patient is not agitated due to unnecessary physical exertion. The patient should be seated for most of the rest period, but may get up to walk to the bathroom.

An increase or decrease in heart rate greater than 15 bpm between the pre- and post-treatment check-ups of visits 1 and 2 will be considered abnormal.

Management as AE: Patients with heart rate greater than 100 bpm or less than 60 bpm.

7.4.5.3 Systemic blood pressure

It is the force exerted by blood flow on the walls of the arteries. Blood pressure is measured with two measurements: systolic (measured when the heart beats, when the pressure is at its highest) and diastolic (measured between heartbeats, when the pressure is at its lowest). When reporting, the systolic is written first, followed by the diastolic.[37]

A calibrated aerobic or mercury sphygmomanometer and a stethoscope will be used for measurement. The measurement should be taken with the left arm and should be done while resting.

An increase or decrease in systolic blood pressure greater than 15 mmHg and in diastolic blood pressure greater than 10 mmHg will be considered abnormal, between the evaluations before and after the application of the investigational product at visits 1 and 2.

Management as AE: Patients with systolic blood pressure of 140 mmHg or more and diastolic blood pressure of 90 mmHg or more.

7.4.5.4 Pupillary diameter

It is the opening in the middle of the iris through which light enters the eyeball. Its diameter is determined by the balance between the pupillary sphincter muscle and the radial fibers of the iris (pupil dilator). Normal values are 2–6 mm. [33]

Since the research product is not a mydriatic, we will consider significant changes in the pupil of the research subjects of > 2 mm (pharmacological mydriasis), whether this change is in photopic or mesopic vision. The measurement will be performed using any of the following tools: OPD III, autorefractors , or topographers that include these measurements. We will only require that the tool used be the same for all patients at the research center and for all their visits. The measurement must always be performed in the same office, under the same artificial lighting conditions, and that the patient be in the room for at least 5 minutes before the measurement.

If there is a change greater than 2 mm between the baseline visit and any of the other measurements, the product will be considered to have produced pharmacological mydriasis, also if there is this change between the examination before and after the application of the investigational product at visits 1 and 2. This change will also be considered an adverse event.

7.4.5.5 Conjunctival hyperemia

It is defined as the simplest reaction of the conjunctiva to a stimulus. A red appearance is observed secondary to vasodilation of the conjunctival vessels of varying intensity. It is graded using the Efron scale. [38]

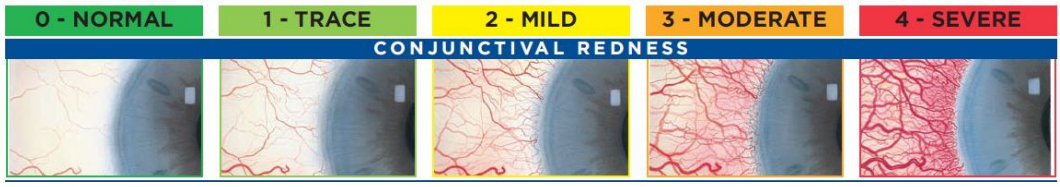


Figure 1. Efron scale for conjunctival hyperemia

Management as AE: Grade 3 and 4 at visits 1, 2 and final will be considered as aggravation and adverse event.

7.4.5.6 Best-corrected visual acuity

Visual acuity (VA) is a test of visual function. Spatial visual acuity is the ability to distinguish separate elements of an object and identify them as a whole. It is quantified as the minimum angle of

separation (located at the nodal point of the eye) between two objects that allows them to be perceived as separate objects.

Snellen notation is described as the distance at which the test is performed divided by the distance at which the letter is vertically equivalent to 5 minutes of arc. Thus, at 6 meters a letter 6/6 (20/20) is equivalent to 5 minutes of arc, a letter 6/12 (20/40) is equivalent to 10 minutes, and a letter 6/60 (20/200) is equivalent to 50 minutes. The Snellen fraction can also be expressed as a decimal (i.e. 20/20 = 1 and 20/40 = 0.5).[34]

VA will be assessed at baseline, without refractive correction, using the Snellen chart. This chart will be placed in a location with adequate natural or artificial lighting and at a distance of 3 m from the subject being assessed. Visual acuity will be measured in the study eye, asking the subject to keep both eyes open and using an occluder to cover the contralateral eye. The subject will read aloud the lines indicated by the evaluator. The line with the smallest letters visible will be recorded by the evaluator as a fraction of the OD VA in the clinical record.

The subject's best refractive correction will then be performed, and the examination will be repeated using the obtained refraction. This result will be reported as best-corrected visual acuity, recorded as a fraction and decimal in both the clinical record and the eCRF .

Management as AE: A decrease of 2 or more lines of vision in BCVA compared to that obtained at the baseline visit will be considered an adverse event.

7.4.5.7 SICCA ocular surface staining

The sequence of ocular surface staining is important for reproducibility and accuracy. Fluorescein should be applied before lissamine green.

Fluorescein staining (TF): A drop of topical anesthetic is instilled in the conjunctival fornix. A second drop is then applied to the tip of the fluorescein strip, allowing it to sit on the strip for 5 seconds to elute the dye, shaking off the excess at the end. A small contact of the strip with the conjunctiva is made in the temporal fornix while the patient looks upward, avoiding damaging the conjunctiva. Corneal fluorescein staining is assessed using a slit lamp with a cobalt blue filter. It is graded according to the Sjögren's Clinical Collaboration Alliance (SICCA) Ocular Staining Grading (OSG). [39]

According to the CTO, grade 0 corresponds to the absence of punctate epithelial erosions (PEEs); grade 1 is defined as the presence of 1-5 PEEs; grade 2 corresponds to 6-30 PEEs; and >30 PEEs will be classified as grade 3. An additional grading point will be added if: 1) PEEs with a diameter of 4 mm are present in the central portion of the cornea; 2) filaments are observed; and 3) confluent staining patches, including linear staining, are observed. [39]See Figure 2.

the grades awarded for corneal staining for OD and OS, respectively, in the file and on the eCFR . The maximum grade per eye is 6.

Lissamine green staining (LGS): After the fluorescein examination, a drop of saline solution is applied to the tip of the lissamine green strip, allowing it to sit on the strip for 5 seconds to elute the dye. A

drop is instilled from the strip into the temporal fornix while the patient looks up, avoiding damaging the conjunctiva. The patient may be asked to blink repeatedly to avoid accumulation in the conjunctival folds. The examination should be performed between 1 and 4 minutes after instillation through a neutral density filter or a red-free filter. It will be graded according to the SICCA CTO. [39]

In CTO, grade 0 is defined as the presence of 0 to 9 lissamine green staining points in the interpalpebral bulbar conjunctiva (with the temporal and nasal portions scored separately); grade 1 is defined as the presence of 10 to 32 points; grade 2 by 33 to 100; and grade 3 by >100 points. Because of the difficulty in counting individual points in a moving eye, any area ≥4 mm ^{2} of confluent points is considered >100 points [39]. See Figure 2.

The PI will record, in the file and in the eCFR , the sum of the grades awarded to the temporal and nasal portions for OD and OS, respectively. The maximum grade per eye is 6.

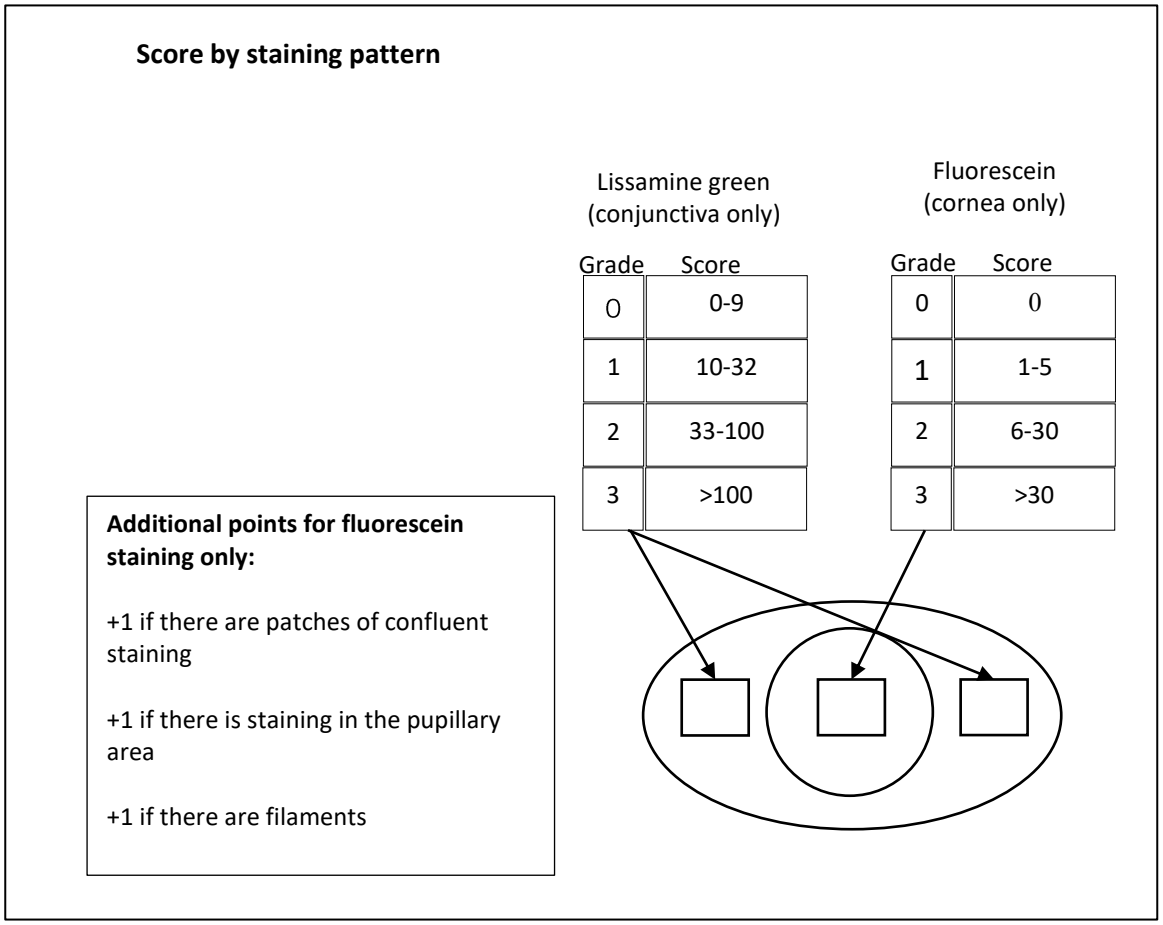


Figure 2SICCA ocular staining grading (Modified from Whitcher et al, 2010)*[34]*

Ocular surface staining using SICCA is determined by the sum of corneal fluorescein staining and conjunctival lissamine green staining. This is shown in Figure 2. The values that can be obtained for

each eye range from 0 to 12, with staining considered abnormal when a value of 3 or higher is reached.

Management as AE: It will be considered an adverse event when the rating is greater than or equal to 3 points.

7.4.5.8 Chemosis

It is defined as conjunctival edema resulting from an inflammatory reaction. It is graded as present or absent. The evaluator will use a narrow 60° beam of light and measure whether the conjunctiva separates from the sclera by ≥ 1/3 of the entire eyelid opening or whether it extends beyond the gray line. [40]

Management as AE: The presence of chemosis will be considered an adverse event.

7.4.5.9 Adverse events

As described in subsection 8.2 Definition of Adverse Event **,** an adverse event is defined as Any adverse medical occurrence in a subject to whom an investigational product is administered, regardless of causal attribution.

The management of adverse events will be carried out in accordance with the provisions of Section 8. Evaluation and management of adverse events and incidents.

The Principal Investigator will record any adverse events that may occur in the study subjects in the corresponding section of the eCRF and will also report them in the clinical record.

For an adequate assessment of adverse events, in addition to the targeted questioning, a Comprehensive Ophthalmologic Evaluation must be performed at each visit . This evaluation consists of: ophthalmologic examination of the eyelids and adnexa; anterior and posterior segments, which are performed during a routine ophthalmologic examination, procedures not specifically included in the study variables. The posterior pole evaluation may be performed with direct or indirect ophthalmoscopy, with or without pharmacological mydriasis, at the discretion of the PI. The fundus will be assessed for abnormalities that could alter the study results. IOP will be measured during this evaluation, using the PI's chosen instrument, and should be measured after the stain evaluation. The results of the evaluation will be recorded in the clinical record. Only those findings that the Principal Investigator considers to be adverse events will be reported in the eCRF .

Adverse events that may occur with PRO-185 are: ocular burning, ocular hyperemia, foreign body sensation, itching, red eye, eye irritation, tearing, little relief of discomfort, blurred vision, burning, superficial punctate keratitis, stinging, mydriasis, increase or decrease in intraocular pressure, release of pigment granules, rebound hyperemia, headache, vertigo, hypertension, hypotension, cardiac irregularities (including tachycardia, bradycardia, among other types of cardiac irregularities), nervousness or excitability, nausea, dizziness, weakness, drowsiness and diaphoresis. [29]

7.4.5.10 Respiratory rate

This is the measurement of the number of breaths per minute taken by research subjects. It is assessed discreetly by observing, without the subject's awareness, the movements of the rib cage, counting each rise/rise of the subject's rib cage as one breath. A range of 12 to 24 breaths per minute is considered normal.

Management as an AE: Any abnormal value will be considered an adverse event. In some cases, the research subject may need to be considered a possible case of COVID-19, and the investigator may need to refer the patient for medical care and withdraw the subject from the research.

7.4.5.11 Body temperature

It is the temperature level of a body. Normal values range from 35°C to 37.4°C. It can be measured using a contact or non-contact thermometer on different parts of the body. Any thermometer calibrated at the research center may be used in the study, as long as the same one is used for all patients and for all their visits.

Management as an AE: Any abnormal value will be considered an adverse event. In some cases, the research subject may need to be considered a possible case of COVID-19, and the investigator may need to refer the patient for medical care and withdraw the subject from the research.

7.5 Description of the procedures or assessments during the study

The various procedures that will be performed during the study are described below. The list may not be in order and could be arranged in the most optimal way according to the needs of the research center.

7.5.1 Signature of informed consent

Procedure that ensures that the research subject has voluntarily expressed his or her intention to participate in this research, after having understood the information given to him or her about the objectives of this research, benefits, discomforts, and possible risks.

7.5.2 Taking a medical history (including ophthalmological and general medical history)

This includes questions about medical history, symptoms, or diagnosed conditions, as well as any medications currently being used, regardless of their route of administration. It includes measurements of body weight and height (somatometry), and a complete ophthalmological evaluation.

7.5.3 Eligibility criteria

It is the evaluation that the patient meets all the inclusion criteria and none of the exclusion or elimination criteria.

7.5.4 Subject code assignment

It is the granting of a code that represents the subject in the study. This code is assigned when the research subject is included in the study.

7.5.5 Adverse events

Described in section 7.4.5.9 Adverse events.

7.5.6 Measuring vital signs

This involves measuring heart rate, respiratory rate, blood pressure, and body temperature. These measurements can be performed with a stethoscope, sphygmomanometer, and mercury or digital thermometer.

Vital signs are described in sections 7.4.5.2, 7.4.5.3, 7.4.5.10 and 7.4.5.11.

7.5.7 Urine pregnancy test

Pregnancy testing will be performed at the baseline and final visits. The researcher must provide the research subject with a female, reproductive-age subject (no natural or induced menopause, defined as 12 consecutive months of amenorrhea) [41]. The patient will be allowed to go to the bathroom and have privacy for the test. After the test is performed, the researcher must verify the result by observing the medical device.

7.5.8 Ophthalmological evaluation

Evaluation of the eyeball, eyelids, eyelashes, and other ocular structures through inspection, slit lamp (biomicroscopy), and palpation (touch). This evaluation includes best-corrected visual acuity, assessment of the integrity of the ocular surface, anterior segment, intraocular pressure, and gonioscopy and posterior segment (fundoscopy).

7.5.8.1 Best-corrected visual acuity

Described in section 7.4.5.6 Best corrected visual acuity.

7.5.8.2 Integrity of the ocular surface

This will be performed using biomicroscopy using the research center's slit lamp. The cornea, conjunctiva, and tear film will be inspected, and ocular staining will be performed with lissamine green and fluorescein (the stains are described in section 7.4.5.7).

7.5.8.3 Anterior segment

This is the evaluation of the anterior segment structures (cornea, conjunctiva, anterior chamber, iris, pupil (including measurement of pupillary diameter), lens, aqueous humor). The evaluation will be performed using a slit lamp.

7.5.8.4 Intraocular pressure

Described in section 7.4.5.1 Intraocular pressure.

7.5.8.5 Gonioscopy

This is the assessment of the iridotrabecular angle by attaching a gonioscopy lens to the patient's cornea. In some cases, the lens will be filled with a gel to improve its fit with the patient's cornea.

The Shaffer system will be used for angle classification.

Table iv. Shaffer classification

Degree	Angular opening	Description	Risk of occlusion
4	45°-35°	Open	Impossible
3	35°-20°	Open	Impossible
2	20°	Narrow	Possible
1	≤ 10°	Extremely narrow	Likely
0	0°	Closed	Occluded

Angle closure or suspected angle closure will be considered if Grade 2 or less is present in more than 180° of the angular circumference.

7.5.8.2 Posterior Segment (fundoscopy)

Also called ophthalmoscopy, this is an examination performed with a light and magnifying glass to observe the fundus (optic nerve and retina) through the pupil. Sometimes, the pupil will need to be dilated to allow a better evaluation of the fundus.

7.5.9 Applying medication during visits

During patient visits, various medications or medical devices may be applied to the ocular surface for ophthalmological examination.

Examples:

- Tetracaine 0.5% ophthalmic solution, used to anesthetize the patient and facilitate measurement of intraocular pressure, application of dyes for staining, and placement of the gonioscopy lens.
- Ophthalmic solution of 0.8% tropicamide and 5% phenylephrine, used to dilate the pupil and better assess the posterior segment.
- PRO-185 ophthalmic solution, an investigational drug, applied at visits 1 and 2.
- Hypromellose 2% ophthalmic solution may be used by the Researcher for gonioscopy assessment.

7.5.10 Delivery of material for the subject

This refers to the provision of the subject's ID card and the subject's diary. The ID card will serve as identification with the treatment assignment number; this card can also serve as an appointment card. The Subject's Diary is used to record the number of times the treatment is administered.

7.5.11 Delivery of the study medication

Once the patient is in the study, the investigational product will be given to them, and the dosage will be explained (one drop four times a day in both eyes).

7.5.12 Evaluation of concomitant medications

This question asks about any medications you are currently taking regularly or have used in the past month. If you have required medication therapy injected into the eye, you should be asked about any medications you have injected in the past 6 months.

7.5.13 Assessment of treatment adherence

This refers to indirectly assessing the number of applications during the period between visits. To assess the approximate number of drops, the medication dropper bottle (concomitant or investigational treatment) can be weighed. The Subject Diary is also reviewed to determine the recorded applications.

7.5.14 Return of study medication

It refers to the return of the product under investigation by the research subject.

7.5.15 Withdrawal from the subject's journal

It refers to the delivery of the subject's diary by the research subject.

7.6 Diagram and schedule of study activities

7.6.1 Study diagram

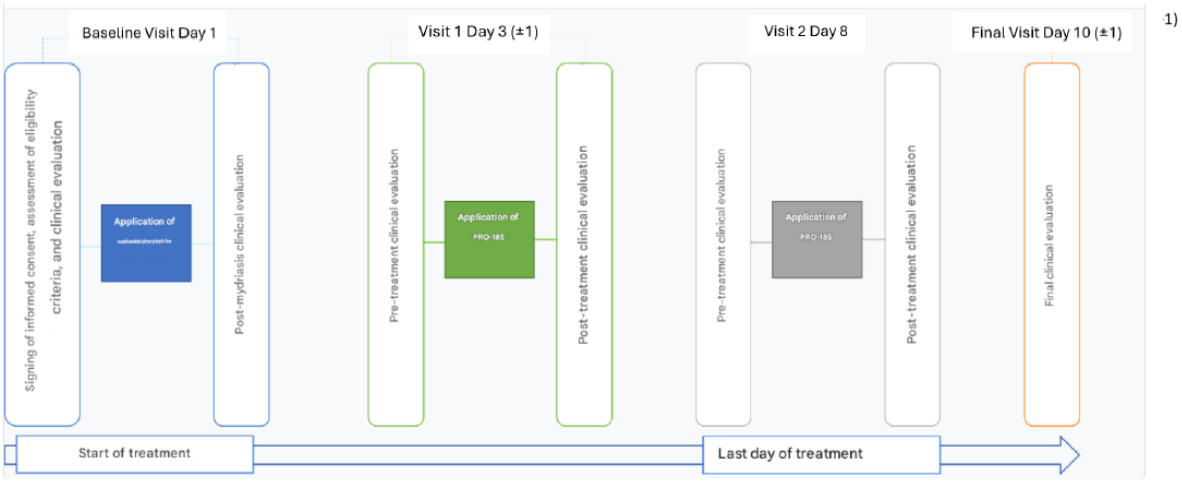


Figure 3Study diagram

7.6.2 Study activity schedule

Table v. Study schedule

Procedures	*Baseline visit Day 1*		*Visit 1 Day 3 (+1)*		*Visit 2 Day 8*		*Final visit Day 10 (+1)*
	Pre-TP	Post - TP	Pre - Tx	Post- Tx	Pre - Tx	Post- Tx	Without treatment
FCI Signature	X						
Medical record	X						
Eligibility criteria	X						
Subject code assignment	X						
Adverse events	X	X	X	X	X	X	X
Heart rate	X	X	X	X	X	X	X
Respiratory rate	X		X		X		X
Blood pressure	X	X	X	X	X	X	X
Temperature (°C)	X		X		X		X
Urine pregnancy test	X						X
AVMC	X		X		X		X
Anterior segment evaluation	X	X	X	X	X	X	X
Pupillary diameter measurement	X	X	X	X	X	X	X
Eye stains	X	X	X	X	X	X	X
Intraocular pressure	X	X	X	X	X	X	X
Gonioscopy	X						
Pharmacological mydriasis (phenylephrine/tropicamide)	X						
Evaluation of the posterior segment	X		X		X		X
Application of PRO-185 during a visit			X		X		
Delivery of material for the subject		X					
Delivery of the study medication		X					

Procedures	*Baseline visit* *Day 1*		*Visit 1* *Day 3 (+1)*		*Visit 2* *Day 8*		*Final visit* *Day 10 (+1)*
	Pre-TP	Post - TP	Pre - Tx	Post- Tx	Pre - Tx	Post- Tx	Without treatment
Evaluation of concomitant medications	X		X		X		X
Adherence assessment			X		X		
Return of study medication						X	
Withdrawal from the subject's diary						X	

PRE-TP: prior to application of 0.8% tropicamide and 5% phenylephrine ophthalmic solution (TP Ofteno®, indicates that the checks will be performed prior to pharmacological mydriasis. Post-TP: post application of the TP Ofteno® solution , indicates that the checks will be performed with pharmacological mydriasis. Pre- Tx : pretreatment, indicates the checks to be performed before the application of the investigational medicinal product. Post-Tx : post-treatment, indicates the evaluations to be performed within 20-40 minutes after the application of the investigational medicinal product. ICF: informed consent form. BCVA: best corrected visual acuity.

7.7 Procedures to be performed per visit

The procedures to be performed at each visit are listed below; these may not be in the optimal order for the Research Center. The researcher should organize them according to their needs, and the needs of the study and sponsor.

In assessing treatment adherence, the subject's diary may be reviewed and/or the dispensed dropper bottles may be weighed.

7.7.1 Baseline visit

- Consent signature informed
- General and ophthalmological clinical history taking
- Measurement of vital signs (temperature, blood pressure, heart rate, respiratory rate)
- Drug evaluation concomitants
- Measurement of best-corrected visual acuity
- Ophthalmological evaluation (before and after pupil dilation)
- Evaluation of pupil diameter
- Ocular staining with fluorescein and lissamine green
- Measurement of intraocular pressure
- Gonioscopy
- Review of eligibility criteria
- Event evaluation adverse
- Pupil dilation
- Delivery of the investigational product

7.7.2 Visit 1

- Pre-application procedures for the investigational product
    - Measurement of vital signs (temperature, blood pressure, heart rate, respiratory rate)
    - Drug evaluation concomitants
    - Measurement of best-corrected visual acuity
    - Assessment ophthalmological
    - Evaluation of pupil diameter
    - Ocular staining with fluorescein and lissamine green
    - Measurement of intraocular pressure
    - Review of continuation criteria
    - Event evaluation adverse

- Post-application procedures for the investigational product
    - Measurement of vital signs (blood pressure and heart rate)
    - Measurement of best-corrected visual acuity
    - Assessment ophthalmological
    - Evaluation of pupil diameter
    - Ocular staining with fluorescein and lissamine green
    - Measurement of intraocular pressure
    - Review of continuation criteria
    - Event evaluation adverse

7.7.3 Visit 2

- Pre-application procedures for the investigational product
    - Measurement of vital signs (temperature, blood pressure, heart rate, respiratory rate)
    - Drug evaluation concomitants
    - Measurement of best-corrected visual acuity
    - Assessment ophthalmological
    - Evaluation of pupil diameter
    - Ocular staining with fluorescein and lissamine green
    - Measurement of intraocular pressure
    - Review of continuation criteria
    - Event evaluation adverse

- Post-application procedures for the investigational product
    - Measurement of vital signs (blood pressure and heart rate)
    - Measurement of best-corrected visual acuity
    - Assessment ophthalmological
    - Evaluation of pupil diameter
    - Ocular staining with fluorescein and lissamine green
    - Measurement of intraocular pressure
    - Review of continuation criteria
    - Event evaluation adverse
    - Return of the research product

- Return of the subject's diary

7.7.4 Final visit

- Measurement of vital signs (temperature, blood pressure, heart rate, respiratory rate)
- Drug evaluation concomitants
- Measurement of best-corrected visual acuity
- Assessment ophthalmological
- Evaluation of pupil diameter
- Ocular staining with fluorescein and lissamine green
- Measurement of intraocular pressure
- Event evaluation adverse
- Urine pregnancy test

7.7.5 Unscheduled follow-up visits

At the request of the subject or study personnel, unscheduled follow-up visits may be conducted to report adverse events or any other situation that warrants it. During these visits, all relevant data on reported adverse events must be collected, and an appropriate management plan must be established, if applicable.

7.8 Data collection

7.8.1 Source documents

Source documents are all written or printed records derived from automated processes (e.g., printouts of laboratory results issued by automated analytical equipment) where information is first recorded and which become part of the subject's permanent medical record. Examples of source documents include medical records, clinical progress notes, laboratory reports, office study reports, nursing notes, follow-up notes, surgical records, etc.

The PI is obligated to accept monitoring of study-related information, audits, review by ethics and research committees, and inspections by the health authority. This obligation implies direct access to source documents.

7.8.2 Electronic forms of data collection

All protocol-related data will be captured via an electronic case report form (eCRF) by research team staff. Protocol-related data should NOT be captured directly into the eCRF , but rather transcribed from the corresponding source document. This procedure allows for monitoring to verify the information captured in the eCRF . It is the researcher's responsibility to ensure that the information is transcribed into the eCRF correctly, completely, and in a timely manner. It is understood that all data captured and submitted via the eCRF for data analysis have been approved by the researcher.

7.8.3 File

The data collected in this database are anonymous (only the subject number is stored along with other relevant information). The software used for data capture and storage meets the traceability

requirements necessary for conducting clinical studies. The collected data will be stored by the sponsor or designated clinical research organization for a period of 10 years. Records of subject number assignment will remain at the participating institutions under the care of the PI or their team and must be maintained for at least 5 years.

8. Evaluation and management of adverse events

8.1 Regulation and standards on adverse events

The registration and reporting of adverse events and incidents will be carried out in accordance with the guidelines established in NOM-220-SSA1-2016 and the international ICH E6 guidelines. [35] [42] [43] [44]

8.2 Definition of adverse event

According to the International Conference on Harmonization (ICH), an adverse event (AE) is any unfavorable medical occurrence in a clinical research subject administered a pharmaceutical product, regardless of causal attribution.[42] [43] [44]

Therefore, an AE may be any of the following: any unfavorable, unintended disease, symptom, or sign (including an abnormal laboratory finding) that is temporally related to the use of a medical product, whether or not considered to be related to that product; any new disease or exacerbation of an existing disease (worsening of the nature, frequency, or severity of a known condition); relapse of an intermittent medical condition (e.g., headache) not present at baseline; any deterioration in a laboratory value or other clinical test (e.g., electrocardiogram [ECG], x-ray) that is related to the symptoms or that results in a change in study or concomitant treatment or discontinuation of study drug.[42] [43] [44]

As defined in the previous paragraph, an adverse event is defined as any event that occurs during treatment with a drug or device. However, the definition can also apply to any undesirable event that occurs during a clinical trial, including behavioral disturbances.[44]

8.3 Use of adverse events as a study safety variable

Measuring the safety of PRO-185 use is paramount to the study, so reporting any undesirable symptoms or illnesses that occur during the course of the study is important, regardless of whether the symptoms are considered related to the investigational treatment.[44]

8.4 Definitions relevant to the classification of adverse events

Severity (serious/non-serious), also called seriousness (serious/non-serious). A serious event is defined as any event that: results in death, threatens life, requires hospitalization or prolongs hospitalization, causes permanent or significant disability or incapacity, causes abnormalities or malformations in the newborn, or other medically significant conditions.

Severity (mild, moderate, or severe). Mild conditions present with minimal symptoms and do not require treatment or discontinuation of the medication; moderate conditions interfere with normal activities without threatening the patient's life, require treatment, and may or may not require discontinuation of the medication; severe conditions interfere with normal activities and require pharmacological treatment and discontinuation of the medication.[35] [42] [43]

Causality. The relationship assigned between the pharmaceutical product and the adverse event: certainly caused by the pharmaceutical product, there is clear evidence of causality, i.e. the adverse event recurs with the administration of the pharmaceutical product; probably caused by the

pharmaceutical product, there is a high suspicion of causality but direct evidence is lacking or it is considered unnecessary or dangerous, i.e. the reaction disappears upon discontinuation of the pharmaceutical product; possibly caused by the pharmaceutical product, there is additional information suggesting that the cause may be due to another pharmaceutical product or disease; unlikely to be caused by the pharmaceutical product, there is a clear explanation for the origin due to the underlying disease or the use of another pharmaceutical product; conditional, there is a lack of data to establish a clear causality; unclassifiable, those for which, once all possible information on the adverse event has been obtained, it remains unclassifiable.[35] [42] [43] [44]

8.5 Researcher Responsibilities

The investigator is responsible for verifying the AE through a history, a relevant physical examination, assessment of progress, and appropriate medical and pharmacological management. The investigator is also responsible for monitoring the AE until it is resolved or resolved, and the patient is discharged, following the definitions established in national and international regulations.[35] [42] [43]

In the event of an AE or any event that puts the health and well-being of the subjects at risk, appropriate medical care will be provided, either at the research center or by referring the subject to the highest-resolution hospital with which the research center has a medical care agreement. The PI will notify the sponsor's clinical monitor, in accordance with the timeframes established in national and international regulations. In the case of serious adverse events, the PI will notify the sponsor and record the corresponding information in the eCRF , and in turn, will inform the IEC and the IC.

The attention of the AE will be carried out according to the event attention diagram (see Figure 4. Adverse event attention).

The sponsor's final report will include adverse event reporting in compliance with current national and international regulations.[35] [42]

If the research subject develops a chronic adverse event during their participation in the study, such as diabetes or arterial hypertension, they will be referred to a healthcare professional for chronic treatment. Follow-up and termination of participation will be in accordance with ICH guidelines.

8.5.1 Recording of adverse events in the electronic case report form

The adverse event registry considers:

- Subject identification information such as: subject number, age, sex, and if applicable, specify the eye.
- Information about the causality of the adverse event, its relationship to the investigational products, or to another study-related drug, as appropriate.
- Information on important dates:
  - Date on which the adverse event occurs.
  - Date on which the Principal Investigator is informed of the same.
  - Date of resolution or outcome, as applicable.
- Information on diagnosis and clinical management.

- Establish the outcome or resolution of the event:
  - Recovered/resolved without sequelae
  - Recovered/resolved with sequelae
  - Not recovered/Not resolved
  - Subject who died due to the adverse event
  - Subject who presented death and it is judged that the research product may have contributed
  - Subject who died and this was not related to the product under investigation,
  - A stranger
- Information about the investigational product or the product associated with the adverse event, incident, adverse event, ADR, or SRAM must be recorded. The essential information to be recorded is the generic name, distinctive name, or code of the investigational product or the product associated with the undesirable clinical manifestation. It will also be necessary to record data concerning the lot number, manufacturing laboratory, expiration date, dose, route of administration, start and end dates of administration and/or consumption, and reason for the prescription, depending on whether it is an investigational product or medication (a protocol in which the subject is currently participating) or a medication that the research subject is taking for the treatment of underlying concomitant diseases or for the management of any transient signs or symptoms that do not correspond to the natural history of the pathology that motivated their entry into the research protocol.
- Indicate whether the adverse event disappears upon withdrawal of the suspected product (which caused the event). Also indicate whether a dose adjustment is made, whether the event changes in intensity or severity, and whether the reaction persists. It is important to indicate whether the AE reappears in subjects who are re-exposed to the product after having been previously discontinued.
- Information regarding concomitant pharmacotherapy. Indicate the generic name, dose, route of administration, start and end dates, and the reason for the prescription, regardless of whether it is in accordance with the prescribing information or the data sheet or if it is used outside of the regulations or as authorized by the local, national, or international regulatory body.
- Information on relevant clinical history. The analysis of the AE considers the information previously described. However, the clinical context in which the adverse event occurs in the participants in the clinical research protocol is of particular interest. Therefore, information about previous conditions, hypersensitivity or allergy symptoms, previous surgical procedures, laboratory tests or imaging examinations the participant has undergone, etc., that the researcher deems appropriate may be mentioned.

8.5.2 Monitoring of adverse events

The Principal Investigator will provide care and follow-up of the adverse event presented by the participant until its outcome, in accordance with the provisions of the following section.

8.5.3 Procedures for a serious adverse event

The adverse event handling process considers the following stages:

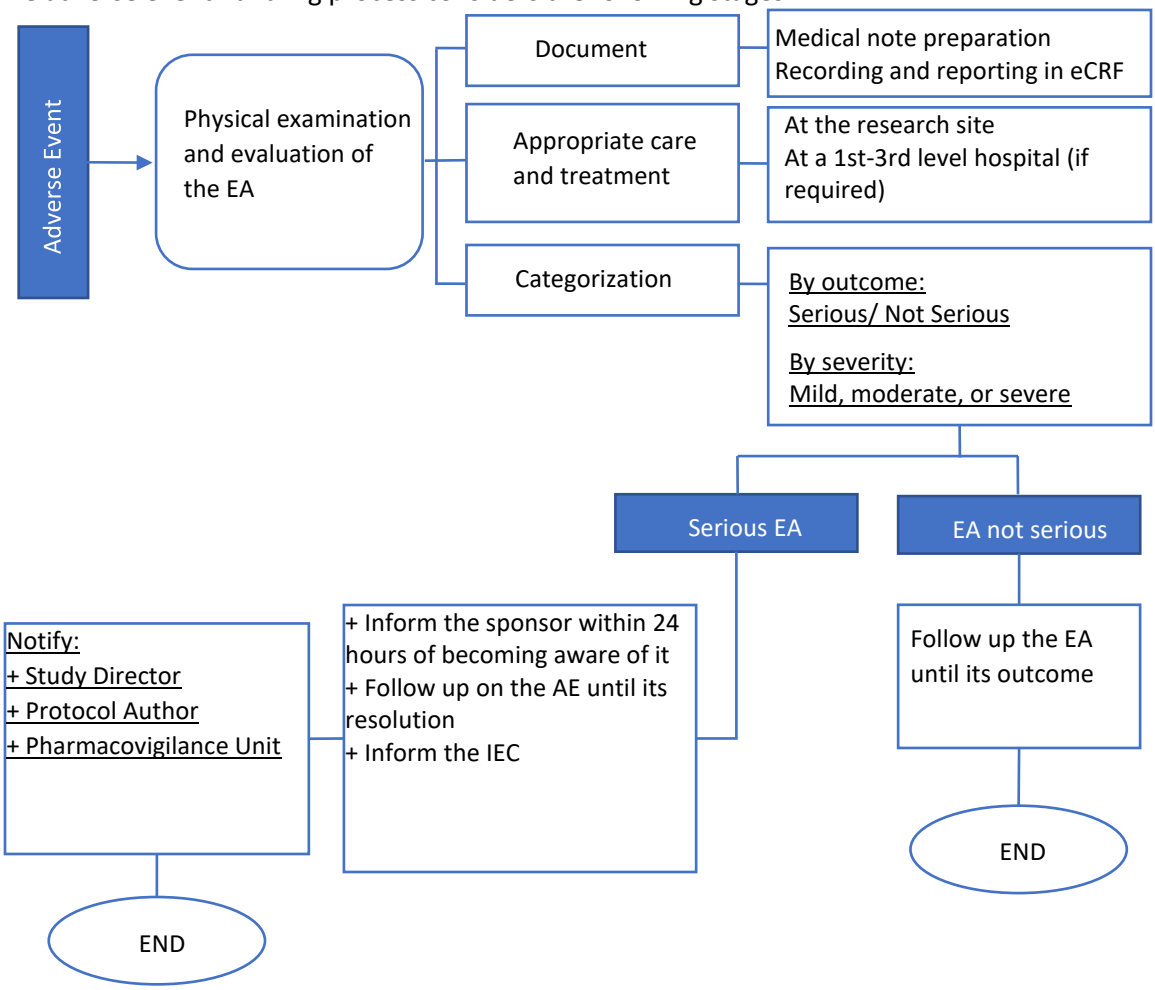


Figure 4Adverse event care

During the development and conduct of this study, undesirable harmful events or adverse reactions/incidents with medical implications may occur in the research subject, which are not necessarily causally related to the investigational products. These harmful phenomena may occur during the use of investigational pharmaceutical products at doses authorized for human use by a local, national, or international regulatory body. However, it may be suspected that the investigational product may cause some unwanted clinical manifestation. AEs, Incidents, Adverse Incidents, ADRs, or SRAMs related to one or more pharmaceutical products may occur during the systematic evaluation of participants (on the days on which the clinical review is scheduled, according to the schedule of activities) or suddenly, in such a way that:

1. The investigator should be the first person to whom the subject notifies that he or she has developed or experienced any clinically harmful phenomena during his or her participation in this study.
2. Based on their clinical judgment, the principal investigator will determine the appropriate course of treatment for the adverse event/reaction based on the relevant

physical examination, history, etc., as well as the analysis of information available in the medical literature and the information provided in the investigator's manual, prescribing information, or the comparator drug's data sheet.

3. This care may be provided at the research center or at the hospital with the highest capacity for treatment. Thus, if the subject is referred by the PI to a hospital, they will be provided care through a referral system. The referral may be through a card identifying the subject as a study participant and linking them to the pre-established agreement with the institution, or through a referral medical note issued by the Principal Investigator. Laboratorios Sophia, SA de CV, will pay the costs for the participating subject's medical care when the adverse event is associated with or related to the investigational product.

4. Taking into account the clinical information collected, either during the care provided at the research center or provided by the treating physician(s) at the hospital, the IP will record the AE in his/her clinical note, stating the seriousness, intensity (mild, moderate, or severe), and relationship to the investigational product.

5. The PI must migrate the relevant data to the eCRF and its respective adverse event section. Serious adverse events must be reported to the study's clinical monitor within 24 hours of becoming aware of them, so that they can then inform the Clinical Team and the UTFLS, and subsequently notify the IEC/CI. Non-serious adverse events will be recorded and appropriately addressed, and the corresponding regulatory body will be informed about the safety profile of the PI or investigational drug in the final clinical trial report.

The recording of the outcome of the AE depends substantially on the Principal Investigator's follow-up of the subject, since most adverse events (see subsection 2.5 Risk-Benefit Assessment and the Investigator's Manual) are expected to be ophthalmic in nature; however, systemic alterations may exist. Therefore, at the investigator's discretion, the participant's withdrawal or continuation will be considered.

8.5.4 Assessment of causality

Causality assessment is the methodology used to estimate the probability of attributing an observed adverse event to a pharmaceutical product. It considers probabilistic categories according to the available evidence and the quality of the information, based on national pharmacovigilance and technovigilance regulations.[35] [45]

An adverse event may or may not be related to the clinical study. A causal relationship means that the intervention caused (or is reasonably likely to have caused) the adverse event. This usually involves a relationship between the timing of the intervention and the adverse event (for example, the adverse event occurred shortly after the research subject received the intervention).[44]

For all adverse events, the Principal Investigator is responsible for examining and evaluating the patient to determine the association of the event with the clinical study and intervention, whether related to experimental treatment, concomitant treatment, surgical procedure, or diagnostic procedures performed during the study.[44]

Accepting that the adverse event is related to the clinical study requires a plausible mechanism of action—that is, a logical sequence between the event and the intervention that caused it. In some

cases, it is helpful to know the opinions of other physicians directly or indirectly involved in the study, as well as whether the patient believes there is a relationship.[44]

The Pharmacovigilance and Technovigilance Unit of Sophia Laboratories (UFTLS) may use the causality categories described by *The* Uppsala *Monitoring Centre to* categorize the probability of the adverse event to the investigational product or to concomitant or used treatments during the visits:[35] [45]

- Definite (certain): A clinical event, including laboratory test abnormalities, that occurs with a plausible time sequence related to drug administration and that cannot be explained by concurrent disease or other drugs or substances. The response to drug withdrawal must be clinically plausible. The event must be definitive from a pharmacological or phenomenological standpoint, using, if necessary, a conclusive rechallenge procedure.[35] [45]
- Probable: A clinical event, including laboratory test abnormalities, that occurs in a reasonable time sequence related to drug administration, is unlikely to be attributed to the concurrent disease or other drugs or substances, and to which withdrawal of the drug produces a clinically reasonable response. Rechallenge information is not required for this definition.[35] [45]
- Possible: A clinical event, including laboratory test abnormalities, that manifests within a reasonable timeframe related to medication administration, but which can also be explained by concurrent illness, or by other drugs or substances. Information regarding medication discontinuation may be missing or unclear.[35] [45]
- Unlikely: A clinical event, including laboratory test abnormalities, that occurs in a time sequence that is unlikely to occur in relation to the administration of the drug and that can be more plausibly explained by concurrent disease, or by other drugs or substances.[35] [45]
- Conditional/Unclassified: A clinical event, including laboratory test abnormalities, reported as an adverse reaction, for which further data are essential for proper evaluation, or additional data are under review.[35] [45]
- Not Evaluable/Unclassifiable: A report that suggests an adverse reaction, but which cannot be judged due to insufficient or contradictory information, and which cannot be verified or completed in its data .[35] [45]

Thus, the degree of certainty to establish the research product as the causal agent of the harmful phenomenon that occurs in the subject of the clinical study can be indicated directly by the Principal Investigator based on his or her clinical experience or through the application of the causality categories described by *The Uppsala Monitoring Centre* . It is important that the researcher and the UFTLS take into account the following arguments in favor of the causal relationship:

a) Strength of association, which refers to the number of cases in relation to those exposed.

b) The consistency of the data, that is, the presence of a common characteristic or pattern.

c) The exposure-effect pattern, which determines the relationship with the site of onset, time, dose and reversibility after suppression.

d) Biological plausibility, which refers to the possible pharmacological or pathophysiological mechanisms involved in the development or presentation of the adverse event.

e) Experimental findings, for example, the appearance of anomalous metabolites or high levels of drug or its biotransformation product.

f) Analogy, which refers to the experience acquired with other related drugs, adverse reactions frequently produced by the same family of pharmacological agents.

g) Nature and characteristics of the data, i.e. objectivity, accuracy and validity of the relevant documentation.[46]

8.6 Unanticipated problems

Unanticipated problems (ANP) are considered situations that pose risks to the participating subjects, generally any incident, experience or result that meets all of the following criteria:

- Unexpected in terms of its nature, severity, or frequency in relation to: 1) study-related documents such as the investigator's manual, study protocol, and informed consent form; and 2) the characteristics of the study population.
- Related or possibly related to your participation in the study (possibly related means that there is a reasonable possibility that the incident or results were caused by study procedures).
- Indication that the research places participants at greater risk of harm (including physical, psychological, economic, or social) than previously recognized.

8.6.1 Reporting unanticipated problems

The PI will be responsible for reporting PNAs to the sponsor, the IC, and the IEC. The report should contain the following information:

- Study identification: protocol title and number, name of the PI and, where applicable, the center.
- Detailed description of the event, incident, experience or outcome.
- Explanation, justification of the reasons why the incident represents a PNA.
- Description of changes to the protocol or corrective actions taken or proposed in response to the NAP.

PNAs that are EAS must be reported to the IEC/ CI and the sponsor within the first 24 hours of the IP becoming aware of it.

Any other PNA will be reported to the IEC/CI and the sponsor within the first 5 business days after the IP becomes aware of it.

9. Study monitoring

The study sponsor is responsible for monitoring the study. Monitoring activities include, but are not limited to: general safety monitoring, general study quality monitoring, study site monitoring, adverse event detection monitoring, reporting and follow-up, monitoring to resolve data entry discrepancies, etc.

Responsibility for monitoring activities and ultimate responsibility for monitoring rests with the sponsor.

The details of the monitoring activities are specified in a separate document from this protocol in a Monitoring Plan.

9.1 Study center monitoring

Monitoring will be conducted at the research center participating in the study. At least one initial visit and one closing visit must be conducted for each center, although one or more follow-up visits may be required between these two mandatory visits.

The initial visit must be conducted before the first participant is enrolled at that center. During this visit, the monitor will verify that the materials to be used during the study have been received and that the personnel involved in study activities have been trained in the study. The monitor will also verify compliance with applicable regulatory requirements and standard operating procedures.

At the follow-up visit(s), the monitor will review the study documents to confirm that the research protocol and applicable standard operating procedures are being followed, that data entry is complete and timely, and that adverse event reporting is being conducted appropriately. At each visit, the monitor will discuss the findings with the investigator and determine the appropriate actions to be taken.

The closing visit will take place at the end of the study, once the last participant at the site has been discharged from follow-up. During this visit, the monitor will verify that the site has all necessary documentation for archiving, that all biological samples have been analyzed, that all PI (used and unused) has been returned to the sponsor, and that all unused materials have been recovered.

Details of monitoring are set out in the relevant plan.

9.2 Audit and quality control

To ensure compliance with GCPs and all applicable regulatory requirements, Laboratorios Sophia, SA de CV may conduct quality assurance audits. Regulatory agencies may also conduct a regulatory inspection of this study.

Details of the audit process are set out separately in an Audit Plan.

9.2.1 Pre-study audit

The research center included in the study will be subject to a feasibility visit prior to center selection, where it will be verified that it meets the minimum requirements indicated by the sponsor.

9.2.2 Audit during the conduct of the study

They may take place at any time before, during, or after the conclusion of the study. If an audit or inspection is conducted, the investigator and the institution must agree to allow the auditor/inspector direct access to all relevant documents and must allocate their time and staff time to the auditor/inspector to discuss the findings and any pertinent issues. If the audit has not been scheduled by the sponsor, the center must notify Laboratorios Sophia, SA de CV immediately.

10. Sample size calculation and statistical analysis

10.1 Sample size calculation

The sample size calculation was based on the study's primary objective: to evaluate the safety and tolerability of PRO-185 ophthalmic solution (naphazoline hydrochloride 0.03% and hypromellose 0.2%) in clinically healthy subjects. To meet the study's primary objective, an estimated 22 subjects will contribute OA to the study.

10.1.1 Calculation methodology

A search was performed on the open access search engines PubMed (pubmed.ncbi.nlm.nih.gov) and Web of Science (webofscience.com), finding until May 2021, 102 results in PubMed and 24 in Web of Science that matched the search keywords: " *Naphazoline Ophthalmic* ". The extraction of this search was generated and the integrated database was reviewed, to eliminate duplicate matches and filter those articles that were in accordance with the objective of the study. After reviewing the *abstracts* (available only in 89 articles), 7 articles were chosen for their complete review (full text), of these, 5 articles were selected to make the proposal for the sample size [47, 48, 49, 50], one of them is a review article [51], this was used only as a reference. See appendix 16.3.

The mean ± standard deviation (SD) sample size used in the selected studies was 24.4 ± 21.24 subjects (range, 6–60). Considering a 20% incidence rate for AEs, these values were entered into an online tool [52]to estimate sample size and power, following specific equations for the calculation [53]. Finally, the calculated value was increased by 20% to account for potential losses.

10.1.2 Size calculation

PRO-185 ophthalmic solution is expected to present in less than 20% of subjects any of the following AEs: increased IOP (>5 mmHg), heart rate changes (>15 bpm), increased systemic blood pressure (>15 mmHg in SBP or >10 mmHg in DBP), pharmacological mydriasis or grade 3 and 4 conjunctival hyperemia, 20 minutes after its application with respect to its initial value.

The sample size was calculated using the equation for a non-superiority proportion [53]. This equation is useful for non-inferiority/superiority tests, if we want to test whether a proportion (p) is not superior to a reference value, p_0 (20% in this case). The idea is that statistically significant differences between the proportion and the reference value cannot be of interest unless the difference is greater than a threshold δ (10% in this case). This type of calculation is useful in clinical studies where the value of δ is chosen based on clinical judgment and knowledge of the subject.

The hypothesis test is:

$$H_0: p - p_0 \leq \delta$$

$$H_1: p - p_0 > \delta$$

Equations

The calculation to estimate the sample size and power was performed using an online tool and following the equations [53]:

$$18 = p(1-0.2)\left(\frac{1.96 + z_{1-\beta}}{p-0.2-0.1}\right)^2$$

$$1-\beta = \Phi(z-1.96) + \Phi(-z-1.96)\,, z = \frac{p-0.2-0.1}{\sqrt{\frac{p(1-0.2)}{18}}}$$

Where:

n is the sample size (18)

p_0 is the reference value (0.20)

Φ is the function of the standard normal distribution

Φ^{-1} is the function of the standard normal distribution

α is the Type I error (1.96)

β is the Type II error, which means that, 1-β (1-0.8) is the power

δ is the test margin (0.10)

According to the previous calculation, 18 subjects are estimated, increasing the calculation by 20% considering possible losses, 22 subjects are estimated to contribute OA to the study. This calculation is in accordance with the average number of subjects used in the clinical studies reviewed in the search performed in PubMed and Web of Science (24 subjects), where the incidence of adverse events was similar to that expected in this study.

10.2 Clinical data management

Clinical data management (CDM) enables the generation of high-quality, reliable, and statistically valuable data. CDM is the process of collecting, cleaning, and managing subject information in a study in compliance with regulatory standards (21 CFR Part 11, ICH, and GCP guidelines). It covers eCFR design , eCFR commenting , database design, *data entry* , validation (*Source Document Verification (* SDV), discrepancy handling (*queries*), medical coding (*medical coding*), extraction (*soft lock*) and closing the database (*hard lock*).[54]

In accordance with roles and responsibilities, multiple users can be created, whose access types to the eCRF can be limited to data entry (principal investigator, PI), medical coding, database design, or quality control . *check*) [54, 55]Discrepancy handling will be based on the flow in Figure 5.

The CDM team will include the following roles:

- Data Manager
- Database Designer/Programmer
- Clinical coder (*Medical coder*)
- Clinical Data Coordinator
- Quality control
- *Data* entry *associate*)

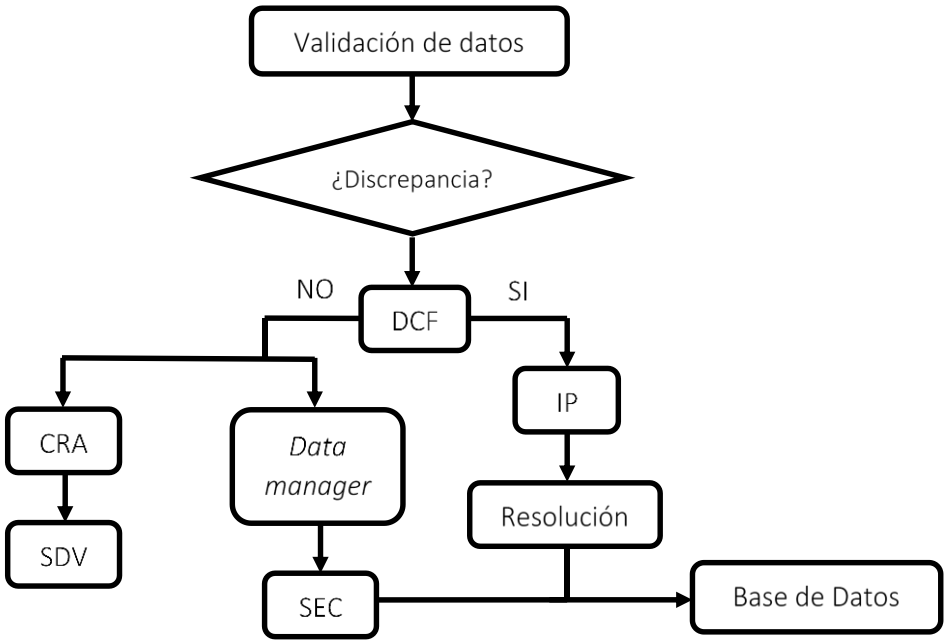


Figure 5Discrepancy management (DCF, medical note; CRA, clinical monitor; SEC, *self-evident) correction*) ***[54]***

10.3 Statistical methodology

The statistical analysis plan was developed considering the evaluation criteria described in the study protocol.

Statistical analysis will be performed by personnel from Laboratorios Sophia, SA de CV. Specialized statistical software will be used (SPSS statistical package, available version, or R software). Coding will be performed using consecutive numbers. Data will be collected and organized in an Excel spreadsheet (Microsoft® Office). The data will then be exported to the specialized statistical software. Variables will be categorized according to their nature (see Table 3). The statistical tests used will follow the corresponding assumptions for nonparametric statistics.

10.3.1 Population analysis

Statistical analysis will be presented to provide a general overview of the subjects enrolled in the study and an overview of the safety and tolerability of the results. Data provided by the research

site will be summarized for this purpose, according to their nature. The Shapiro-Wilk test will be performed to test whether the quantitative data are normally distributed.

The results of the quantitative variables will be presented as measures of central tendency: mean, standard deviation, maximum, and minimum. Changes in pupil diameter, BCVA, and body temperature will be expressed as continuous variables. Changes in IOP, HR, systemic blood pressure, TVL, and TF will be expressed as discrete variables.

The results of nominal and ordinal qualitative variables will be presented as frequencies, proportions, and/or percentages. For these, 2 x 2 frequency tables will be constructed. All percentages will be presented with one decimal place.

The level of difference considered significant will be an alpha (α) of 0.05 or less. The triangulation between the type of variable and the measurements is shown in Table 6 .

Table vi. Triangulation of concepts

Variable type	Variable	A1	A2	B1	B2	C1	C2	C3	C4	D1	D2	D3	D4	E1	E2
Selection															
A1	Demographics	DT													
A2	Medical history/Selection criteria		DT												
Basal															
B1	Vital signs		B	B		B		B	T				TM		
B2	Comprehensive ophthalmological evaluation		DTB		TB	B									
Security															
C1	PIO				B	B							TM		
C2	HR, SBP, DBP			B			B						TM		
C3	Pupillary diameter				B			B					TM		
C4	Conjunctival hyperemia				T				T				T		
Secondary Safety Outcome															
D1	AVMC				B					B			TM		
D2	Corneal and conjunctival stains				B						B		TM		
D3	Chemosis				T							T	T		
D4	Incidence of AE			TM	TM	TM	TM	TM	T	TM	TM	T	TM	TM	TM
Exploratory															
E1	FR			B									TM	B	
E2	Body temperature			B									TM		B

Abbreviations: BCVA, best-corrected visual acuity; B, bivariate analysis; D, descriptive statistics; AE, adverse event; HR, heart rate; RR, respiratory rate; M, multivariate analysis; DBP, diastolic blood pressure; SBP, systolic blood pressure; IOP, intraocular pressure; T, 2x2 contingency table.

10.3.2 Safety and tolerability analysis

10.3.2.1 Analysis for primary variables

The analysis of safety and tolerability outcomes will be conducted in the safety population, defined as all subjects who received at least one dose of the investigational product (PRO-185), regardless of their adherence to the protocol (intention-to-treat, ITT, population).

Statistical analysis for the primary quantitative variables will be estimated using the Wilcoxon signed-rank test for a sample for the difference in measurements before and after treatment for visits 1 and 2, while the baseline visit (pre-PT) will be compared against the final visit, once the values have been adjusted with respect to their baseline within each individual.

For conjunctival hyperemia, the X^2 *binomial test* or Fisher's exact test will be used for expected values less than 5.

10.3.2.2 Analysis for secondary variables

For the analysis of these variables, the same primary analysis will be performed as long as the necessary measurements are available.

10.3.3 Exploratory analysis

The analysis of exploratory variables, FR and body temperature, will be performed on those subjects who complete their participation without deviations from the study protocol (per-protocol population, PP).

10.4 Procedure for handling missing data

An imputation procedure for missing data is not contemplated.

11. Ethical considerations

11.1 Approval of committees

This study will be conducted in accordance with the standards of the Declaration of Helsinki, World Medical Association 2013. Nuremberg Code ; Nuremberg Judgment by the International Tribunal at Nuremberg , 1947. Belmont Report, National Commission for the Protection of Subjects of Biomedical and Conduct Research, 1979. It will be conducted in accordance with the scientific and technical requirements necessary for the registration of medicines for human use of the International Conference on Harmonization (*The International Council for Harmonisation* (ICH) Guideline for Good Clinical Practice. International Ethical Guidelines for Biomedical Research Involving Human Subjects of the Council for International *Organizations of Medical Sciences of Medical Sciences ,* CIOMS, 2002). International Ethical Guidelines for Epidemiological Studies of the Council for International *Organizations of Medical Sciences of Medical Sciences* , CIOMS, 2008). The Research Ethics Committee and the Research Committee will evaluate the protocol before conducting the study and will issue their approval or any possible modifications for its implementation. These Committees must be notified of any significant changes to the protocol. In addition to the above, the current regulations of the regulatory authority must also be complied with.

The sponsor's authorized personnel will submit the essential documentation of the research project for evaluation by the Research Ethics Committees, Research Committees, and when applicable, to the Biosafety Committee: research protocol, informed consent form, researcher's manual, subject material, as well as other additionally requested documents, in accordance with the local, national or international requirements applicable by regulatory entities.

The study will not be initiated at the research center without the confidentiality agreements and financial proposals from each of the principal investigators, duly signed, and without having previously obtained the favorable opinion and/or approval of the corresponding Research Ethics Committees, Research Committees, and, where applicable, the Biosafety Committee.

The study will not begin without meeting the relevant local, national, or international regulatory requirements and obtaining the appropriate health authorization.

The study is considered to be research with greater than minimum risk, in accordance with the Regulations of the General Health Law on Health Research, Title Two, Chapter I, Article 17, Section III, published in the Official Gazette on January 6, 1987.

11.2 Amendments to the protocol

The amendment process will be relevant when there is a need to make any changes to a document that is part of the research project or protocol, due to changes in the methodological structure, replacement of the principal investigator, or the identification of risks to the research subjects.

Documents that may be amended include: the protocol, informed consent letter, researcher's manual, subject documents, measurement scales, and activity schedule.

Any amendment must be approved by the sponsor and/or the principal investigator. The amended document(s), once reviewed and approved by the Research Ethics Committee and the Research Committee or, when applicable, by the Biosafety Committee (entities that issued the initial favorable opinion for the conduct of the research), will be sent for authorization by the regulatory entity.

Amendments that substantially modify the protocol or impose additional or different risks to research subjects must be approved by the aforementioned Committees. It is the investigator's responsibility to take measures in situations requiring immediate action to prevent unnecessary harm to study participants.

The principal investigator is responsible for communicating to the Research Ethics Committee any amendments to the protocol that could affect the rights, safety, or well-being of the research participants. They must also report any situation or new knowledge that indicates an increased risk to the participants, the premature termination or suspension of the study, the reasons for this, and the results obtained to that point. They must also report the conclusion of the study upon completion of the research protocol.

11.3 Early termination of the study

The study may be temporarily suspended or terminated prematurely if there is sufficiently reasonable cause. Written notification documenting the reason for the suspension or early termination must be provided by the party executing the suspension. The PI must promptly inform the study participants, the IC, and the IRB, providing the reasons.

Situations in which suspension or early termination of the study will be considered include, but are not limited to:

1. The presence of serious adverse events in more than 10% of participants in a study group.
2. The regulatory authority considers it to be a security alert.
3. The Sponsor determines it for its convenience or eventualities such as: financial support, manufacturing errors, etc.
4. The determination of unexpected risks to participants that are significant or unacceptable.
5. Obtaining new relevant safety information.
6. Insufficient adherence to protocol requirements.
7. The data obtained are not evaluable or are not sufficiently complete.
8. The determination that the primary objective has been achieved.
9. The determination of futility.

In the event of suspension, the study may be resumed once the situations that led to the suspension have been resolved, provided this justification is sufficient for the sponsor, IC, IEC, and regulatory authorities.

11.4 Informed consent

The FCI contains complete and understandable information about the study and the investigational product, in accordance with current applicable regulations and Good Clinical Practices.

The FCI will be considered a source document and will be filed as such. The site's principal investigator is responsible for ensuring that all new versions of the informed consent form undergo the appropriate approvals (the same ones that the original informed consent form underwent) and that the most current approved version is presented to the study subjects.

11.4.1 Obtaining

Informed consent must be obtained before the subject undergoes any procedure indicated in the protocol. For this purpose, the informed consent form must be signed.

Written consent documents will incorporate the elements of informed consent described in the Declaration of Helsinki and the ICH Guidelines for Good Clinical Practice and will be in compliance with all applicable laws and regulations.

The PI, or the study staff delegated by him or her, will provide the potential participant with all information regarding the characteristics of the study, its potential benefits, risks, objectives, and procedures.

This information will be provided in a language understandable to the subject. The subject will be explained that they have the right to discontinue their participation in the study at any stage, without affecting their relationship with the researcher and/or their future participation. Informed consent will be presented to the potential participant; they must have sufficient time to review each and every aspect mentioned above. Any questions they may have will be clarified by the person responsible for obtaining informed consent.

Once the participant agrees to participate in the study, he or she must sign and date the informed consent letter in the presence of two witnesses, whether or not related to the study subject. These witnesses will participate in the informed consent process and sign, confirming that the process was carried out prior to any study procedure, that the study information was clearly explained, and that any questions were clarified.

In the event that a subject is illiterate, acceptance will be with his or her fingerprint, and in the event that the subject is not capable of providing adequate written informed consent, a “legally authorized” representative of the subject may provide such consent for the subject in accordance with applicable laws and regulations.

Likewise, the PI, or the study staff delegated by him, must sign and date this consent.

The FCI must be signed in duplicate by all involved; one copy will be filed in the researcher's folder and the other will be given to the participant. The PI or designated staff member must document the process of obtaining Informed Consent through a detailed, accurate, and contemporaneous medical note, specifying the signed version, the date the document was signed, and how the process was carried out.

11.4.2 Special considerations

The procedures that will be performed during the conduct of the study do not pose any additional risk that should be considered apart from the procedures listed in the informed consent.

11.4.3 Modifications to informed consent

Any changes to the FCI constitute an amendment to this document and must be submitted for approval to the regulatory committees and authorities.

Such amendments may be implemented only after obtaining written approval from the Research Ethics Committee and the Regulatory Body (as applicable), except for an amendment that is required to eliminate an immediate danger to the study subjects.

re-consent process must be conducted for each subject affected by the amendment under the same conditions as those described above, in order to promptly communicate the new information contained in the document. The subject will be given a signed original of the amendment, and the researcher will retain the second original.

11.5 Confidentiality

All documents and information provided to the research center by the sponsor are strictly confidential. The PI expressly agrees that the data regarding his or her professional and clinical experience, provided to the sponsor in paper form and stored electronically, are solely for use in connection with his or her activities with the clinical study sponsor, in accordance with Good Clinical Practice.

The PI agrees that he and his team members will use the information only within the scope of this study, to carry out the protocol. This agreement is binding as long as the confidential information has not been publicly disclosed by the sponsor.

The clinical study protocol provided to the PI may be used by the PI and his or her team to obtain informed consent from the subjects for the study. The clinical study protocol, as well as any information derived from it, must not be disclosed to other parties without the sponsor's written authorization.

The PI will not disclose any information without the prior written consent of Laboratorios Sophia, SA de CV, except to representatives of the Competent Authorities, and only at their request. In the latter case, the researcher is obligated to inform Laboratorios Sophia, SA de CV before disclosing the information to these authorities.

The PI will complete and maintain a subject selection log, as well as the identification and enrollment list of each subject participating in the study. The researcher agrees to grant on-site access to the auditor and/or representatives of the Competent Authorities. The information will be treated in compliance with professional secrecy.

In the eCRF and all communications related to study subjects, they will be identified only by their study subject identification number, either the screening number or the allocation number. The information collected in this study will be exchanged between the sponsor and the research site and must be treated confidentially. The Health Authority, the IRB, the IC, the sponsor, the monitors/auditors, and third-party auditors will be the only bodies authorized to review the study documentation. If publications arise from this research project, under no circumstances will they contain information about the identification of the study subjects. If the study results are published, no personal information about the study subjects will be revealed.

The protection of personal data will be in accordance with the corresponding current regulations.

11.6 Conflict of interest

The independence of the study's conduct and results from any actual or perceived external influences is critical. Therefore, any current conflict of interest of any person playing a role in the design, conduct, analysis, publication, or any other aspect of this study will be declared. Furthermore, those with a perceived conflict of interest will be asked to manage it in a manner appropriate to their participation in the study.

11.7 Access to information

The final study database will be the property of Laboratorios Sophia, SA de CV, and access to it will be restricted. The PI will not have access to it except with prior written authorization from the sponsor.

Any information obtained that is relevant to the safety of the subjects participating in the study must be immediately shared with the research center, so that the study subjects can be notified.

11.8 Auxiliary and post-study care

Once the study is completed and adverse events are closed in accordance with section 8, the sponsor will not extend care to the research subject.

12. Biosecurity aspects

NO BIOSECURITY IMPLICATIONS

This protocol, entitled: “Phase I clinical study to evaluate the safety and tolerability of PRO-185 ophthalmic solution applied in clinically healthy subjects,” and number: SOPH185-0521/I, HAS NO BIOSAFETY IMPLICATIONS, since no infectious biological material will be used; pathogenic strains of bacteria or parasites; viruses of any type; radioactive material of any type; genetically modified animals and/or cells and/or plants; toxic, hazardous, or explosive substances; or any other material that puts the health or physical integrity of the research center staff or research subjects at risk, or affects the environment. It is also declared that no cell, tissue, or organ transplant procedures, or cell therapy, will be carried out in this project, nor will laboratory, farm, or wildlife animals be used.

13. Publication Policy

13.1 Final report

Once the statistical analysis is completed, the final report will be written with the results obtained, by the Medical Management Department Team of Laboratorios Sophia, SA de CV. This report will be prepared following the recommendations of the ICH E3 *Step 4 Guide.*

13.2 Communication of results

Regardless of the results of the study, Laboratorios Sophia, SA de CV, is committed to communicating the final study report to the principal investigators and COFEPRIS. These results will also be shared with the research committee and the IEC. The PI will be responsible for communicating the results to the research subjects.

Laboratorios Sophia, SA de CV will retain at all times the rights to the publication and dissemination of the information contained herein.

13.3 Publication of results

Laboratorios Sophia, SA de CV, acting as the sponsor of the study, assumes full responsibility for its role and retains exclusive ownership rights to the study results, which it may use as it sees fit.

The PI agrees not to publish or communicate data collected from the study, unless prior written agreement is obtained from Laboratorios Sophia, SA de CV. Any manuscript derived from the data obtained with this protocol must be reviewed by the sponsor before any attempt to submit it for publication in any journal or scientific conference.

However, if the sponsor is in the process of filing a patent application on the results of the study, the sponsor may delay publication or communication of the results of the study until the date of registration or when it deems appropriate.

The assignment of authors for publications derived from this research will be the sole responsibility of the sponsor. Researchers invited to participate as authors must express their authorization. All researchers who will participate as authors may review the article prior to submission and are free to make comments and suggestions within the first 15 calendar days of receipt.

14. Financing and insurance

14.1 Compensation to study participants

Subjects participating in the study may receive financial compensation for their participation. Compensation may vary depending on the activities required during each visit.

14.2 Insurance for study participants

Subjects participating in the study will sign the informed consent form, which specifies that Laboratorios Sophia, SA de CV agrees to pay for immediate treatment resulting from injuries or illnesses caused by the investigational products until resolved, in accordance with medical judgment.

15. Bibliography

[1] H. Cronau, RR Kankanala, and T. Mauger, "Diagnosis and management of red eye in primary care," *Am Fam Physician,* vol. 81, no. 2, pp. 137-144, 2010.

[2] C. Wirbelauer, "Management of the red eye for the primary care physician," *Am J Medicine,* vol. 119, pp. 302-306, 2006.

[3] J. Kuryan, "The itchy eye: diagnosis and management of ocular pruritus," 2010. [Online]. Available: http://www.aao.org/publications/eyenet/201002/loader.cfm?url=/commonspot/security/getfile.cfm&PageID=121911.

[4] DA Palay and JH Krachmer, Primary Care Ophthalmology, 2nd ed., Philadelphia: Elvevier Mosby, 2005, pp. 89-102.

[5] JD Bartlett and SD Jaanus, Clinical Ocular Pharmacology, 5th ed., Butterworth-Heinemann, 2008, pp. 247, 249, 268, 379, 514, 560, 578.

[6] OA Olajuyin, OS Obimakinde, OS Olatunya and TG Olajide, "Red eye: the red herring of otolaryngologic diseases," *Journal of Otology & Rhinology,* vol. 6, no. 6, 2017.

[7] S. Narayana and S. McGee, "Bedside diagnosis of the red eye: A systematic review," *The American Journal of Medicine,* vol. 128, pp. 1220-1224, 2015.

[8] PS Braich and R. Kuriakose, "How to triage the red eye: considerations for non-ophthalmic providers," *Journal of Advanced Practices in Nursing,* vol. 2, no. 1, 2016.

[9] J. Oporto, F. Zuazo, G. Valdes and X. Contreras, «Red eye syndrome in primary care: diagnosis and management,» *Scientific Journal of the Chilean Society of Family and General Medicine of Chile,* 2016.

[10] C. Snyder and LO Hosten, "Over-the-Counter ocular decongestants in the United States- Mechanism of action and clinical utility for management of ocular redness," *Clinical Optometry,* vol. 12, pp. 95-105, 2020.

[11] Code of Federal Regulations Title 21. Part 349 -- Ophthalmic Drug Products for Over-The-Counter Human Use, "Code of Federal Regulations," [Online]. Available: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=349.75. [Last access: June 2, 2021].

[12] J. Bartlett, Ophthalmic Drug Facts, 25th ed., Walters Kluwer Health, 2013, pp. 64, 75-77, 83, 349-350.

[13] Rite Aid Corporation, "Lubricant / Redness Reliever - Naphazoline Hydrochloride and Hypromellose solution / drops," [Online]. Available: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=8ff03df6-32d6-425d-8bd7-838bb6885391. [Last access: May 24, 2018].

[14] P. Hurwitz and JM Thompson, "Uses of naphazoline (Privine) in ophthalmology," *Arch Ophthalmol,* vol. 43, no. 4, pp. 712-717, 1950.

[15] MJ Ogidigben, TC Chu and DE Potter, "Naphazoline-induced suppression of aqueous humor pressure and flow involvement of central and peripheral alpha (2)/(1) receptors," *Exp Eyes Res,* vol. 72, no. 3, pp. 331-339, 2001.

[16] SC Sweetman, Martindale: The complete drug reference, Grayslake, IL: Pharmaceutical-Press, 2009, p. 1565;2144.

[17] General Directorate of Medicines, Supplies, and Drugs of the Ministry of Health of the Republic of Peru, "Technical Report No. 17 - 2008 Naphazoline 0.05% x 5 mL," [Online]. [Last accessed: 2019].

[18] Toxnet, “Naphazoline hydrochoride,” [Online]. Available: https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~YKNeqS:1 .

[19] TJ Liesegang, “Viscoelastic Substances in Ophthalmology,” *Survey of Ophthalmology,* vol. 34, no. 4, 1990.

[20] I. Toda, N. Shinozaki and K. Tsubota, "Hydroxypropyl Methylcellulose for the treatment of severe dry eye associated with Sjögren's Syndrome," *Cornea,* vol. 15, no. 2, pp. 120-128, 1996.

[21] GA Burdock, "Safety assessment of hydroxypropyl methylcellulose as a food ingredient," *Food Chem Toxicol,* vol. 45, no. 12, pp. 2341-2351, 2007.

[22] GP Kamatou, I. Vermaak, AM Viljoen and BM Lawrence, “Menthol: a simple monoterpene with remarkable biological properties,” *Phytochemistry,* 2013.

[23] A. Robbins, M. Kurose, BJ Winterson, and ID Meng, “Menthol activation of corneal cool cells induces TRPM8-mediated lacrimation but not nociceptive responses in rodents,” *Invest Ophthalmol Vis Sci,* vol. 53, pp. 7034-7042, 2012.

[24] L. Jones, LE Downie, D. Korb, JM Benitez-del-Castillo, R. Dana, SX Deng, PN Dong, G. Geerling, RY Hida, Y. Liu, J. Xu, JS Wolffsohn and JP Graig, "TFOS DEWS II Management and Therapy Report," *The Ocular Surface,* pp. 580-634, 2017.

[25] X. Xu, N. Yu, Z. Nai , *Y.* 249, pp. 1503-1510, 2011.

[26] Laboratorios Sophia, SA de CV, «Final report of results of preclinical research protocol No. NACOVSNAFAVSPLACPRESEP14. "Comparative preclinical study on the safety and clinical toxicity of pro-171 ophthalmic solution when compared to N ophthalmic solutions,» Laboratorios Sophia, SA de CV, Zapopan, Jal., 2014.

[27] L. Baiza-Durán, J. Álvarez-Delgado, Y. Contreras-Rubio and J. Medrano-Palafox, «Study of the safety and acute toxicity of a topical ophthalmic reformulation of Naphacel Ofteno developed by Laboratorios Sophia SA de CV applied to the ocular surface of healthy New Zealand albino rabbits,» Laboratorios Sophia, Zapopan.

[28] L. Baiza-Durán, J. Álvarez-Delgado, Y. Contreras-Rubio and J. Medrano-Palafox, «Safety and tolerance study of a topical ophthalmic reformulation of Naphacel Ofteno developed by Laboratorios Sophia SA de CV applied to the ocular surface of healthy human volunteers,» Laboratorios Sophia, Zapopan.

[29] ASHP, ASHFS Drug Information, Bethesda, MD: American Society health-System Pharmacists, Inc, 2015.

[30] Sato Pharmaceutical Co., Ltd, “Sato Clear - naphazoline hydrochloride,” Sato Pharmaceutical Co., Ltd., Tokyo, Japan, 2019.

[31] Rite Aid Corporation, "Lubricant / Redness Reliever - naphazoline hydrochloride and hypromellose solution / drops," Rite Aid Corporation, 2014.

[32] Novartis, “New Zealand Data Sheet,” Novartis, Auckland, NZ, 2018.

[33] F. Leon-Sarmiento, D. Prada and C. Gutiérrez, “Pupil, pupillometry and pupilography,” *Acta Beurol Colomb,* vol. 24, pp. 188-97, 2008.

[34] J. Whitcher, C. Shiboski, S. Shiboski, A. Heidenreich and e. al., "A simplified quantitative method for assessing keratoconjunctivitis sicca from the Sjogren's syndrome international registry," *Am J Ophthalmol,* vol. 149, pp. 405-15, 2010.

[35] NO Mexicana, *NOM-220-SSA1-2016, Pharmacovigilance Facilities and Operations.,* CDMX: Official Gazette of the Federation. , 2017.

[36] Pan American Health Organization, "Heart Rate," bvs Virtual Health Library.

[37] National Cancer Institute, “Blood pressure,” NCI Dictionaries.

[38] N. Efron, “Grading scales for contact lens complications,” *Ophtalmic Physiol Opt,* vol. 18, pp. 182-186, 1998.

[39] J. Whitcher, C. Shiboski, S. Shiboski, A. Heidenreich and e. al., "A simplified quantitative method for assessing keratoconjunctivitis sicca from the Sjogren's Syndrome International Registry," *Am J Ophthalmol,* vol. 149, pp. 405-415, 2010.

[40] European Group of Graves Orbitopathy, «Eugogo,» ETA, [Online]. Available: http://www.eugogo.eu/_downloads/clincial_evaluation/CHEMOSIS-GO.pdf. [Last accessed: 11 April 2016].

[41] A.P. Torres Jiménez and J.M. Torres Rincón, "Climaterium and Menopause," *UNAM School of Medicine Review,* vol. 61, no. 2, pp. 51–58, 2018.

[42] International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, “Clinical Safety Data Management: Definitions and Standards for Expedited Reporting E2A,” ICH Harmonized Tripartite Guideline, 1994.

[43] IInternational Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, "General Considerations for Clinical Trials," ICH Topic E8, 1998.

[44] Good Clinical Practice, “Participant Safety & Adverse Events,” gcp.nidatraining.org.

[45] The Uppsala Monitoring Centre, “Medicine Safety Monitoring,” World Health Organization, 2001.

[46] RH Meyboom, AC Egberts, IR Edwards, YA hekster, FH de Koning and FW Gribnau, “Principles of signal detection in pharmacovigilance,” *Drug Safety,* vol. 16, pp. 355-365, 2997.

[47] P. Hurwitz and J. Thompson, “Uses of naphazoline (Privine(R)) in ophthalmology,” *Arch Ophthal,* vol. 43, no. 4, pp. 712-7, 1950.

[48] M. Anelson, M. Allansmith, and M. Friedlaender, “Effects of topically applied ocular decongestant and antihistamine,” *Am J Ophthalmol,* vol. 90, no. 2, pp. 254-7, 1980.

[49] M. Abelson, S. Brutus, J. Weston, and B. Rosner, “Tolerance and absence of rebound vasodilation following topical ocular decongestant usage,” *Ophthalmology,* vol. 91, no. 11, pp. 1364-7, 1984.

[50] B. Nayak, K. Kishore and S. Gupta, “Evaluation of oxymetazoline and naphazoline in bening red eyes: a double blind comparative clinical trial,” *Indian J Ophthalmol,* vol. 35, no. 4, pp. 190-3, 1987.

[51] L. Hosten and S. C, "Over-the-counter ocular decongestants in the United States-Mechanisms of action and clinical utility for management of ocular redness," *Clin Optom (Auckl),* vol. 12, pp. 95-105, 2020.

[52] HyLow Consulting LLC, “Power and Sample Size,” 2013–2021. [Online]. Available: http://powerandsamplesize.com. [Accessed May 10, 2021].

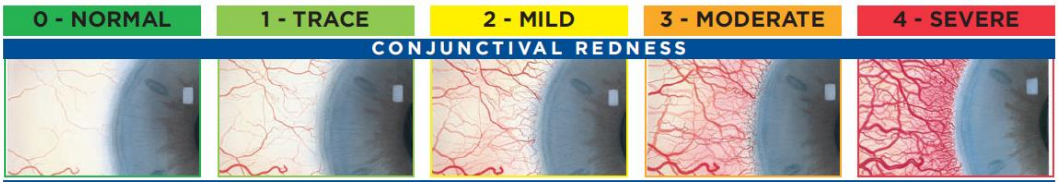
[53] S. Chow, J. Shao and H. Wang, from *Sample size calculations in clinical research* , 2nd ed., New York, Chapman and Hall/CRC biostatistics series, 2008.

[54] B. Krishnankutty, S. Bellary, N. Kumar and L. .. Mooddahadu, "Data management in clinical research: an overview.," *Indian J Pharmacol,* vol. 44, no. 2, pp. 168-72, 2012.

[55] L. Su, "Clinical data management: current status, challenges, and future directions from industry perspectives.," *OA J Clin Trials,* vol. 2, pp. 93-105, 2010.

16. Annexes

16.1 Efron scale for conjunctival hyperemia



16.2 SICCA Ocular Staining Grading (modified from Whitcher et al, 2010)

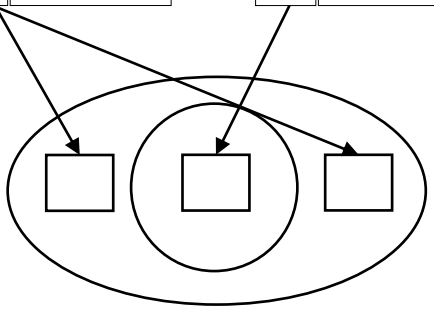
Score by staining pattern

Lissamine green (conjunctiva only)

Grade	Score
0	0-9
1	10-32
2	33-100
3	>100

Fluorescein (cornea only)

Grade	Score
0	0
1	1-5
2	6-30
3	>30



Additional points for fluorescein staining only:

+1 if there are patches of confluent staining

+1 if there is staining in the pupillary area

+1 if there are filaments

16.3 Review of articles for sample size calculation

Reference	Summary	Subjects	Treatment
Hurwitz P, Thompson JM. Uses of naphazoline (privine ®) in ophthalmology. Arch Ophthal . 1950; 43(4): 712-7. doi :10.1001/archopht.1950.00910010723008.	Naphazoline hydrochloride has been widely used as a nasal decongestant. Considering its potential uses as an ocular decongestant, it was decided to evaluate its properties in certain clinical conditions. A series of experiments were conducted to evaluate the effects of naphazoline on intraocular pressure, pupillary size, and the accommodation and condition of ocular blood vessels in **60 patients** (both eyes). The **0.1% naphazoline solution** was well tolerated by the eye. A mild to moderate burning sensation was experienced after instillation. Occasionally, itching occurred, and some patients with lightly pigmented irides reported transient blurred vision.	60	Naphazoline 0.1%
Abelson MB, Allansmith MR, Friedlaender MH. Effects of topically applied ocular decongestant and antihistamine. Am J Ophthalmol . 1980; 90(2): 254-7.	In two independent studies involving **25 subjects** each, **0.05% naphazoline** produced significant whitening of histamine-induced red eyes and ocular itching (but did not prevent itching). Antazoline significantly inhibited itching (but not red eyes). The combination of naphazoline and antazoline produced significant whitening and inhibition of itching in all histamine-challenged eyes. The combination of both drugs was more effective than either component individually in preventing redness. The antihistamine/vasoconstrictor combination was equally effective for itching.	25	Naphazoline 0.05%
Abelson MB, Butrus SI, Weston JH, Rosner B. Tolerance and absence of rebound vasodilation following topical ocular decongestant usage. Ophthalmology . 1984; 91(11): 1364-7. doi : 10.1016/s0161-6420(84)34140-9.	Two commercial preparations of topical ophthalmic vasoconstrictors were evaluated for their bleaching ability, duration of action, tolerability, and rebound vasodilation in **11 healthy volunteers** . Both treatments, **naphazoline hydrochloride 0.02%** and tetrahydrozoline hydrochloride 0.05%, significantly decreased initial redness after a single application (Part I); however, naphazoline produced significantly more bleaching than tetrahydrozoline. Only naphazoline maintained bleaching after 10 days (Part II). The level of	11	Naphazoline 0.02%

Reference	Summary	Subjects	Treatment
	redness remained significant from baseline for 8 hours after a single instillation for both vasoconstrictors and for 6 hours after multiple administrations of naphazoline. The decrease in tetrahydrozoline's effectiveness after the 10-day study period may encourage its overuse. No vasoconstrictor produced rebound vasodilation after discontinuation.		
Nayak BK, Kishore K, Gupta SK. Evaluation of oxymetazoline and naphazoline in benign red eyes: a double blind comparative clinical trial. Indian J Ophthalmol . 1987; 35(4): 190-3.	The efficacy of oxymetazoline and **naphazoline (0.01%) was evaluated** in **20 patients** with red eye. The decongestive effect of oxymetazoline 0.01% on days 3 and 7 was 66.66% and 80.55%, compared with 22.22% and 50% for naphazoline 0.01%. Oxymetazoline showed a significant improvement in conjunctival symptoms such as itching, foreign body sensation, tearing, and burning with naphazoline compared with oxymetazoline. No ocular toxicity or AEs were observed during the study.	20	Naphazoline 0.01%
Hosten LO, Snyder C. Over-the-Counter Ocular Decongestants in the United States - Mechanisms of Action and Clinical Utility for Management of Ocular Redness. Clinical Optometry , 2020; 12: 95 – 105.	This review provides a current, clinically relevant summary of the mechanism of action, efficacy, and safety of available OCT decongestants for the reduction of ocular redness caused by minor irritations. Currently, the OCT products marketed in the US include tetrahydrozoline 0.05%, **naphazoline 0.012% to 0.03%** , and brimonidine 0.025%. All 3 agents are adrenergic receptor agonists but vary in their receptor-binding properties: tetrahydrozoline is a selective α1-adrenergic receptor agonist, naphazoline is a combination α1/α2 receptor agonist, and brimonidine is a selective α2 receptor agonist. These OCT decongestants produce vasoconstriction of conjunctival blood vessels, resulting in a rapid reduction of ocular redness. In general, reported ocular AEs are minimal, mild, and transient, with no significant systemic AEs. However , ocular decongestants with α1-adrenergic receptor agonist activity have been associated with loss of effectiveness after continued use (tachyphylaxis) and rebound redness upon discontinuation. In clinical studies,	146	Naphazoline 0.012 to 0.03%

Reference	Summary	Subjects	Treatment
	brimonidine has not been associated with tachyphylaxis, and rebound redness has been rarely reported.		