Excelencia en oftálmicos

Protocol:

SOPH201-0521/I

Title: Phase I clinical study, to evaluate the safety and tolerability of the PRO-201 ophthalmic solution, prepared by Laboratorios Sophia, S.A. de C.V. on the ocular surface of ophthalmologically and clinically healthy subjects

Information about the molecule under study
Generic name: Atropine Sulfate 0.01%
Distinctive name: PRO-201
Indication: Delayed progression of myopia Delayed progression of myopia

Protocol Information
Study Phase: I
Version: 1.0
Release Date: 10-ago-21

This protocol has been carried out in accordance with the principles of the Declaration of Helsinki and will be carried out in accordance with Good Clinical Practices and in compliance with the ICH guidelines and current local legislation.

Sponsor: Laboratorios Sophia, S.A. de C.V.

Content

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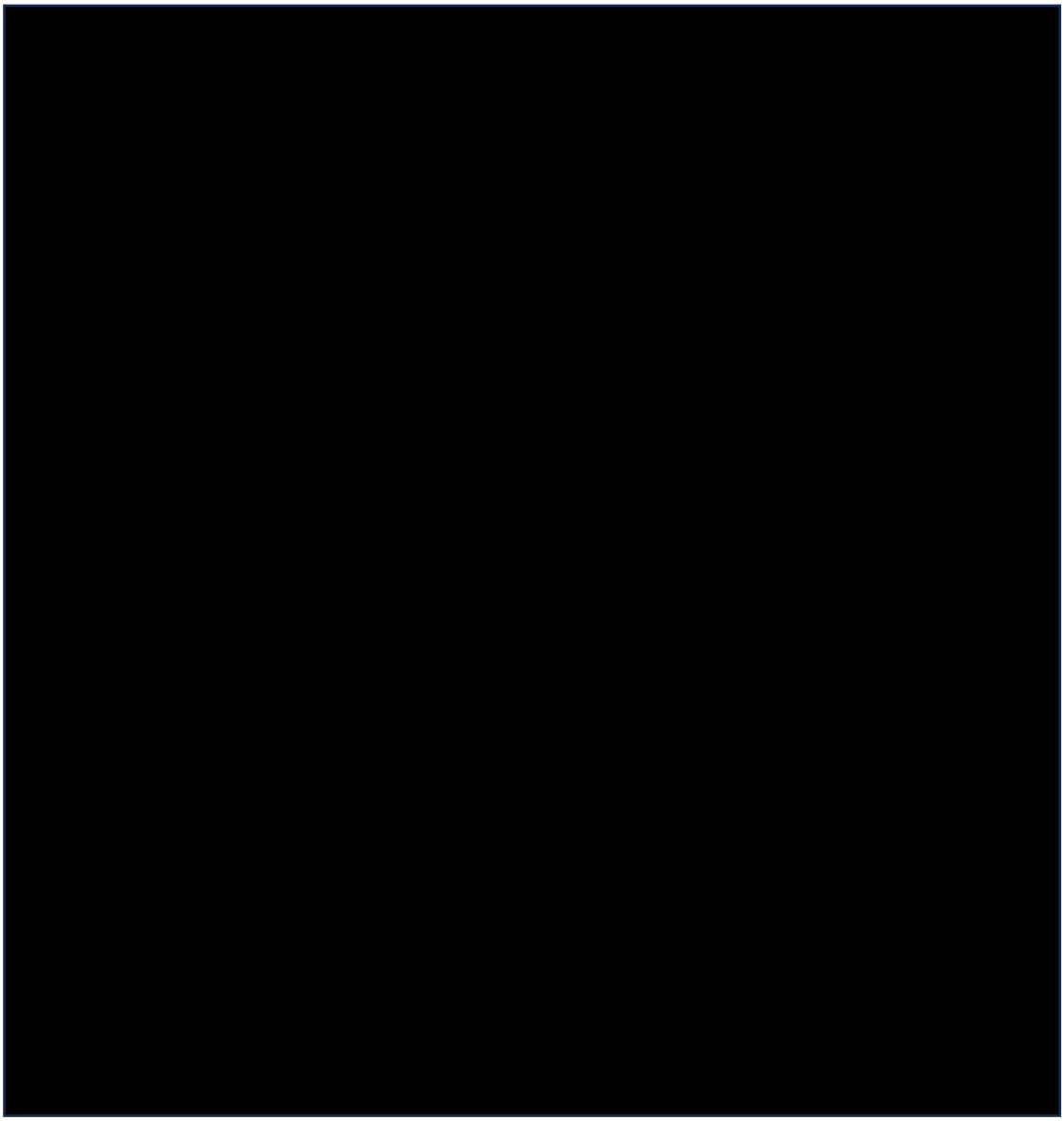
Responsible for the study

The administrative structure of the sponsoring party, corresponding to Laboratorios Sophia, S.A. de C.V., is shown in Table 1and Figure 1. Responsible for the study.

Signature Page

From the sponsor



Investigator Agreement

I agree to conduct this clinical study according to the design and guidelines of this protocol, in accordance with its provisions. I declare that I will conduct the study in accordance with the standards of Good Clinical Practice, and I will report all information or data in accordance with the protocol, particularly any adverse event. I will also handle 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 it is prohibited from sharing it with any third party, which is not involved in the approval, supervision, or conduct of the study. I will ensure that I take the necessary precautions to protect the information from loss, inadvertent disclosure, or access by unauthorized third parties.

Name: *[Write full name of the researcher*	**Signature**
Title: Principal Investigator	**Date**
Name of the center: [*Insert Name of Study Site]*	
Geographic location (city/state/country) *[Enter the geographical data of the center]*	

List of abbreviations

Ad	Adherence
AO	Both eyes
VA	Visual acuity
NVA	Near visual acuity
BCVA	Best-corrected visual acuity
NBCVA	Near Best Corrected Visual Acuity
GCP	Good clinical practice
CDM	*Clinical Data Management*
REC	Research Ethics Committee
CR	Research Committee
COFEPRIS	Federal Commission for the Protection against Sanitary Risks
ESR	Eye Staining Rating
SD	Standard deviation
IUD	Intrauterine device
EA	Adverse Event
eCFR	*Electronic case form report*
USA	United States of America
HR	Heart rate
ICF	Informed consent form
RF	Respiratory rate
H_0	Null hypothesis
H_1	Alternate hypothesis
ICH	International *Council for Harmonisation*
ICMJE	*International Committee of Medical Journal Editors*
ECI	Eye Comfort Index
PI	Principal Investigator
ITT	By *intention to treat*
BPM	Beats per minute
Mmhg	Millimeters of mercury

NOM	Official Mexican Standard
WHO	World Health Organization
BDP	Diastolic blood pressure
RP	Research product
PIO	Intraocular pressure
PNA	Unanticipated problems
PP	Population by protocol
ADR	Adverse drug reaction
NCTR	National Clinical Trials Registry
SDV	Source *document verification*
SICCA	Sjögren*'s international collaborative clinical Alliance*
SPSS	Statistical package for the social sciences
SADR	Suspected adverse drug reaction
FS	Fluorescein staining
TRPL	Tear film rupture time
SLG	Staining with Lysmine Green
UFTLS	Pharmacovigilance and Technovigilance Unit of Sophia Laboratories
CV	Current version
x2	Chi-square
°C	Celsius degrees

1. Protocol Summary

1.1 Synopsis

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

Phase I clinical study, to evaluate the safety and tolerability of the PRO-201 ophthalmic solution, prepared by Laboratorios Sophia, S.A. de C.V. on the ocular surface of ophthalmologically and clinically healthy subjects</td></tr>
<tr><td>Study Number:
SOPH201-0521/I</td><td>Date of creation:
28/May/2021</td></tr>
<tr><td>Protocol version:
1.0</td><td>Release Date:
Aug/10/2021</td></tr>
<tr><td>Therapeutic indication:
Adjuvant in treatment to delay the progression of myopia.</td><td>Use:
Myopia</td></tr>
<tr><td>Estimated duration of the study (from the first visit of the first patient to the preparation of the final report):
6 months</td><td>Clinical Development Phase: I</td></tr>
<tr><td colspan="2">Objectives:

Main objective:

• To evaluate the safety and tolerability of the PRO-201 formulation manufactured by Laboratorios Sophia S.A. de C.V. on the ocular surface of ophthalmologically and clinically healthy subjects.

Specific objectives:

• Primary
o To evaluate the safety of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers, through the incidence of unexpected adverse events (AEs) related to the investigational product (PI).</td></tr>
</table>

- To assess the tolerability of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers using the incidence of photophobia.

- Secondary
  - To evaluate the safety of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers, by evaluating the pupillary diameter by the OPD scan.
  - To evaluate the safety of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers, using the incidence of expected PI-related AEs.
  - To evaluate the tolerability of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers, by evaluating the best corrected near visual acuity (CVA) with the ETDRS primer.

- Exploratory
  - To assess the safety of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers, by assessing best-corrected visual acuity (BCVA).
  - To evaluate the safety of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers, by assessing intraocular pressure (IOP).
  - To evaluate the safety of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers, by evaluating corneal and conjunctival staining with fluorescein and lysmine green.
  - To assess the safety of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers, by assessing vital signs (heart rate and blood pressure).
  - To evaluate the tolerability of PRO-201 ophthalmic solution by means of the Ocular Comfort Index (ILO) score.

Hypothesis:

H0= The PRO-201 ophthalmic solution is not safe in its ophthalmic application as it has an incidence of unexpectedly related adverse events (AEs) greater than 10% and is not tolerable as it presents an incidence of photophobia greater than 15% of the study population.

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

H1= PRO-201 ophthalmic solution is safe in ophthalmic application with an incidence of unexpected, related adverse events (AEs) of less than 10% and is tolerable with an incidence of photophobia of less than 15% of the study population.

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

Study Design:

Phase I Clinical Trial, Controlled, Non-Comparative, Open-label, Single-Center

Number of subjects (planned and analyzed):

Number of planned subjects: 29 evaluable subjects (both eyes)

Diagnosis and main inclusion criteria:

- Ophthalmologically and clinically healthy subjects.

Selection criteria:

Inclusion criteria:

- Be clinically healthy
- Can voluntarily give their signed informed consent (CRF)
- Be able and willing to comply with scheduled visits, treatment plan, and other study procedures
- Be between 18 and 35 years old.
- No history of contact lens use
- Women of childbearing potential must ensure continued use (initiated ≥ 30 days prior to signing the CRF) of hormonal contraceptive or intrauterine device (IUD) use during the study period
- Have a better far-corrected visual acuity of 20/30 (logMAR 0.2) or better in both eyes.
- Have a better corrected visual acuity close to 20/25 (logMAR 0.1) or better in both eyes.
- Have vital signs within normal parameters.
- Have an IOP ≥10 and ≤ 21 mmHg.

Exclusion criteria:

- Be a user of topical ophthalmic products of any kind.
- Allergy or known intolerance to ingredients in atropine eye drops and other derivatives of muscarinic antireceptor agents.
- Being a user of medicines, or herbal products (plant extracts, infusions, naturopathic preparations, homeopathics, etc.), by any other means of administration.
- For women: being pregnant, breastfeeding, or planning to become pregnant within the study period.
- Have participated in any investigational clinical study 90 days prior to inclusion in the present study.
- Have previously participated in this same study.
- Who are unable to follow the lifestyle considerations described in Annex 1.
- Have a history of any chronic-degenerative disease, including diabetes and high blood pressure.
- Have an active inflammatory or infectious disease at the time of study entry.
- Have unresolved injuries or trauma at the time of study entry.
- Have a history of any type of eye surgery.
- Having undergone surgical, non-ophthalmologic procedures within the past 3 months.
- Be or have an immediate family member (e.g., spouse, parent/legal guardian, sibling, or child) who is employed by the research site or sponsor, and who is directly involved in this study.

Elimination criteria:

- Withdrawal of the letter from the FCI.
- Presentation of a serious adverse event related or not to the investigational product, which at the discretion of the PI and/or sponsor could affect the patient's ability to continue with the study procedures safely.
- Non-tolerability or hypersensitivity to any of the compounds used during the tests (fluorescein, lysmine green, tetracaine).
- No tolerability or hypersensitivity to the investigational drug.
- Adherence < 90% determined by the subject's diary

Research Product (PI):

- PRO-201 . Atropine Sulfate 0.01%. Zapopan, Jalisco, Mexico. Ophthalmic solution Laboratorios Sophia, S.A. de C.V
- Dosage: 1 drop once a day (QD) in the evening, in both eyes (AO).
- Route of administration: Ophthalmic

Duration of treatment:	**Duration of the subject in the study:**
14 days	21 days

Evaluation criteria:

Primary outcome variables

- Incidence of unexpected PI-related AEs (Time of Assessment [TE]: days 7, 16 and 21)
- Incidence of IP-related photophobia. (TE: 7th, 16th and 21st)

Secondary outcome variables:

- Changes in pupillary diameter (ET: day 7 and 16).
- Incidence of expected PI-related AEs (TE: days 7, 16, and 21).
- Changes in visual acuity (TE: day 7 and 16).

Exploratory variables:

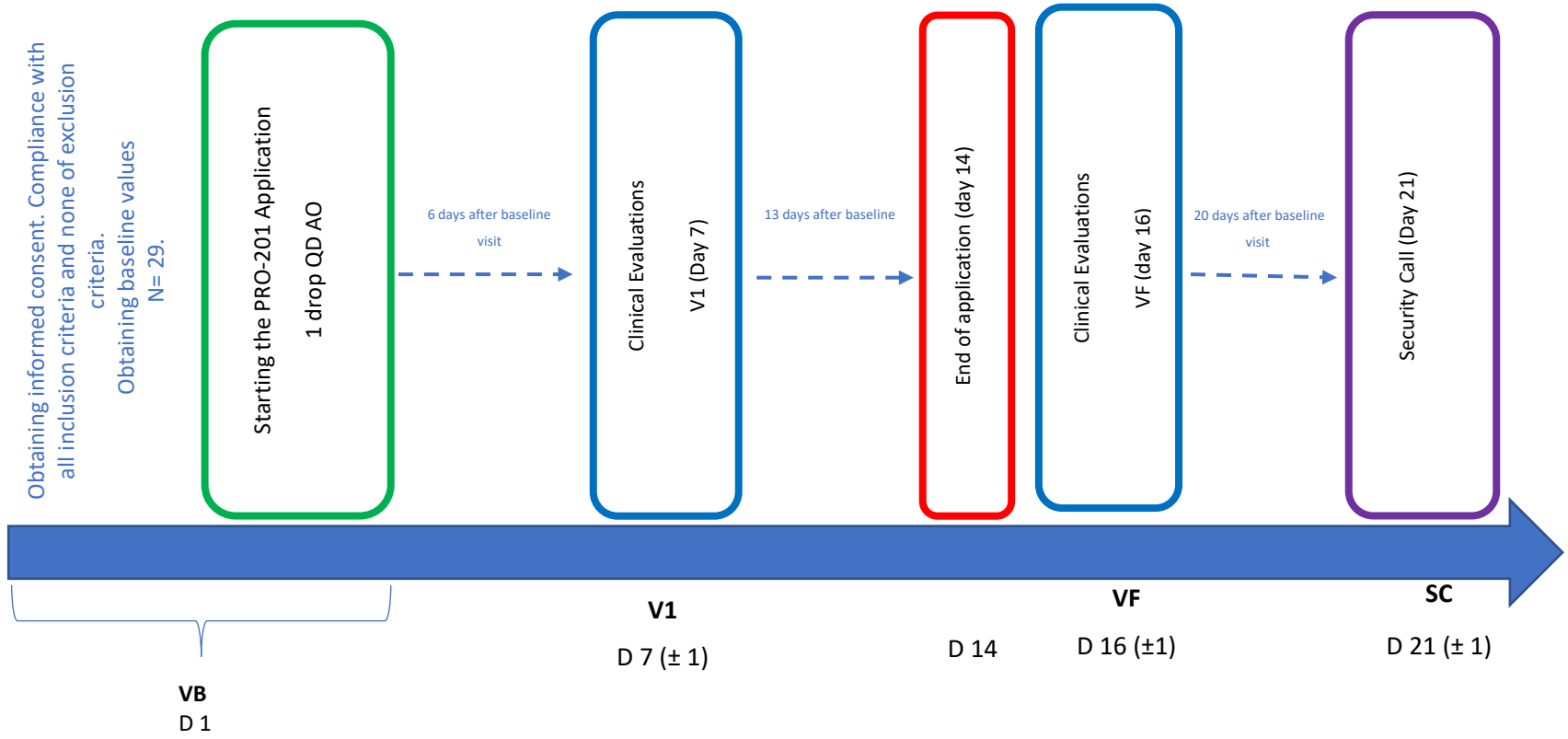
- Changes in the AVMC (TE: day 7 and 16).
- Intraocular pressure (IOP) changes (ET: day 7 and 16).
- Changes in corneal and conjunctival staining with fluorescein and lysmine green (ET: day 7 and 16).
- Changes in vital signs (heart rate and blood pressure) (ET: day 7 and 16).
- Changes in the OCI (TE: day 16).

Statistical methodology

The analysis of the data collected in the study will be carried out using the SPSS version 19.0 statistical package. For continuous and discrete quantitative variables, data will be expressed by

measures of central tendency: mean, standard deviation, and ranges. Qualitative data will be presented as frequencies and percentages. The statistical analysis to rule out differences will be carried out by means of non-parametric statistics. The Kolmogorov-Smirnov statistic will be used for a sample and the Mann-Whitney statistic (when applicable). The analysis for qualitative variables will be performed by means of Pearson's X^2 (Chi-square) test or Fisher's Exact test (when applicable). For all analyses, a $p < 0.05$ and an alpha of 0.05 (two-tailed) will be considered significant.

1.2 Study diagram



BV= Basal visit

VF= Final visit. Evaluation of outcome variables.

QD= Once a day

In parentheses () is the allowed window period.

V1 = Visit 1

SC = Safety Call

AO= Both eyes

1.3 Subject Timeline

Procedures	VB D 1	V1 D 7 ± 1	VF D 16 ± 1	SC D21 ± 1
Informed Consent Form (FCI) Signature	X			
Medical history	X			
Eligibility Criteria	X			
Concomitant Drug Evaluation	X	X	X	
Urine pregnancy test	X		X	
Vitals signs	X	X	X	
Best Corrected Visual Acuity (BCVA)	X	X	X	
Best Corrected Near Visual Acuity (BCNVA)	X	X	X	
Pupillary diameter measurement	X	X	X	
Evaluation of fluorescein staining and lysmine green	X	X	X	
PIO	X	X	X	
Comprehensive ophthalmological evaluation	X	X	X	
Adverse Event (AE) Assessment	X	X	X	X
Subject Code Assignment and Research Product (PI)	X			
Eye Comfort Index (ECI)	X		X	
Delivery of the PI and initiation of intervention	X			
Daily subject delivery	X			
Adherence assessment		X	X	
Return / evaluation of the subject's diary			X	
Return of PI			X	

2. Introduction and background

2.1Theoretical framework

Myopia is a refractive error of the eye in which light rays converge on a focal point in front of the retina, instead of converging on the retina itself, making it difficult to focus well on distant objects. In progressive myopia, this defect occurs due to an exaggerated growth of the eyeball that causes a progressive thinning of all layers of the eye, with the retina being the most affected. [1]

Myopia is one of the five priorities for the World Health Organization (WHO) in the initiative "*Vision 2020: The Right to Sight*" [2]. It is estimated that half of the world's population will be myopic by 2050. [3]

Although the exact etiology of myopia remains difficult to explain, it appears to have both genetic and environmental components, making prevention and treatment challenging and individualized. Stopping the progression of myopia has the potential to positively affect quality of life and eye health.[4]

The initial problem with myopia is that patients who suffer from it do not see far away, but what is really important Limiting the progression of myopia is explained by the increased risk of blindness with ≤ −6 diopters and/or ≥ 26 mm axial length (macular neovascular membrane, macular chorioretinal atrophy, retinal detachments, glaucoma, etc.). [5] [6]

Myopia usually appears between the ages of six and eight and progresses on average diopter per year until the age of 15 or 16, but there are patients who continue to increase for practically their entire lives. [1]

We are facing an increase in myopia globally; in the United States of America, 42% of the population is myopic, a percentage that in 1970 was 25%. In some areas of Asia such as Taiwan, Singapore, and Hong Kong, young adults have myopia rates of 80 to 90%. [7]

There are different studies that show that the most effective way to slow down the progression of this type of myopia is the use of topical antimuscarinic drugs. Among the antimuscarinic drugs, atropine eye drops have been confirmed to be the safest and most effective treatment. [8]

Atropine is the most studied and used non-selective antimuscarinic agent to stop the progression of myopia. The first references, in German and English publications, to the use of atropine in the treatment of myopia date back to the 1970s in the 1970s. [9, 10]

The ATOM studies (for its acronym in English *Atropine for the Treatment of Myopia*) they have used atropine in different concentrations (1%, 0.5%, 0.1% and 0.01%) for a period of up to 5 years, demonstrating a reduction in the progression of myopia; as well as in the increase of axial length. Finding a small percentage of adverse effects in the population studied. [11, 12, 13, 14]

The effect of decreasing the progression of myopic refractive error using 0.01% atropine is currently recognized. However, today 0.01% atropine is only marketed in Asia and magisterial preparations are made in American countries such as Canada. In clinical trials, patients are provided with dilutions prepared with artificial tears. Although there are ophthalmologists who make this dilution in their clinics, this practice can present risks of dosage, stability and asepsis if it is not carried out under adequate conditions. [15]

2.2 Background on the investigational product

We do not have clinical studies of an ophthalmic solution containing 0.01% atropine sulfate so far. However, we have the experience in clinical studies with atropine sulfate at concentrations higher than PRO-201.

2.2.1 Preclinical study to evaluate the feasibility of a 1.0% atropine sulfate-induced model of dry eye in albino rabbits New Zealand

Fifteen healthy New Zealand albino rabbits were given a 1% atropine sulfate solution three times daily for 14 days in both eyes, and five other rabbits served as a control group, as part of a preclinical study to evaluate the feasibility of a dry eye model. In the results of the study regarding the histopathological analysis of eyeballs, all the results of the 40 eyes studied were within normal limits, finding only slight changes such as those related to tissue preservation/fixation and no evidence of any pathological alteration. This is relevant because, together with the IOP levels evaluated (found within normal parameters) and the absence of AEs reported in this study, the histopathological result confirms the safety profile of the currently marketed product. [16]

2.2.2 Clinical study of safety, tolerance and mydriatic efficacy of a topical ophthalmic reformulation of 1% atropine sulfate

Open-label, single-center, observational, prospective clinical study included thirty healthy volunteers. The volunteers administered a drop of 1% atropine sulfate in reformulation once a day in a single dose and in only one eye, and the effect was assessed one hour after application. Biomicroscopy was performed at baseline and one hour after application, evaluating the presence or not of conjunctival hyperemia, conjunctival secretion, ciliary injection, chemosis, and corneal surface, as well as Rose Bengal and Fluorescein stains, grading on a scale of 0= absent, 1= mild, 2= moderate, and 3= severe, according to the staining of the ocular surface and the pupillary diameter, both horizontally and vertically. Of the 30 patients included in this study, there was no presence of drug residues or significant alterations of the conjunctival surface.
Regarding mydriatic efficacy, pupillary dilation of up to twice the basal size was observed, both in the vertical diameter and the horizontal diameter. Regarding tolerance and safety, only 9 patients (30%) reported mild burning, with an average of 0.3 on a scale of 0 to 3. In the same area, there was also no uptake of fluorescein or Rose Bengal on the ocular surface. Based on the results presented, it can be concluded that 1% atropine sulfate (Atro Ofteno®) in reformulation has a good mydriatic efficacy

one hour after its application, as well as being safe and well tolerated when applied to the ocular surface of healthy volunteer patients. [17]

2.3 Background on the investigation

2.3.1 From the research question

Is the application of PRO-201 ophthalmic solution safe and tolerable in clinically healthy subjects?

The importance of the question that precedes this paragraph is that Laboratorios Sophia S.A. de C.V. does not have clinical studies with products identical to the PRO-201 solution, although there are with similar products (different concentration and excipients).

2.4 Risk-benefit assessment

2.4.1 Known potential risks

Formulations containing atropine sulfate at the same concentrations as PRO-201 are products that are marketed in Asia, as well as in compounding formulations in other countries in the Americas and Europe. The risks of using this product in specific populations are described below, however, the use of this drug in the study will be in a population group without the alterations or characteristics described below. To Concentration at 0.01% The potential risks are: photophobia, mydriasis, difficulty reading, allergic eye reactions (atopic dermatitis, allergic conjunctivitis and other allergic reactions), eye irritation, burning eyes, eye pain, tearing, foreign body sensation, dry eye, hyperemia and/or red eye, blurred vision, eye itching; Systemic adverse events include: headache, drowsiness, dizziness, tachycardia, flushing, restlessness, irritability, dry skin, dry mouth, skin rash and decreased alertness. [8] [6] [18] [19] [20] [21] [22] [23] [24]

2.4.2 Known potential benefits

The benefit in the target population is to serve as an adjuvant in the treatment to delay the progression of myopia. But for the study population, as it is a phase I study, it will be a healthy population. Therefore, a direct benefit for each individual is not expected, and the benefit provided by the research will be the[11, 12, 13, 14, 25] to know the behavior of the formulation; Have more data on their safety to be able to prevent or warn of unwanted effects on users or problems with their tolerance.

2.5 Problem statement

The effect of decreasing the progression of myopic refractive error using 0.01% atropine is currently recognized. However, today 0.01% atropine is only marketed in Asia and magisterial preparations are made in American countries such as Canada. In clinical trials, patients are provided with dilutions prepared with artificial tears. Although there are ophthalmologists who make this dilution in their clinics, this practice can present risks of dosage, stability and asepsis if it is not carried out under adequate conditions. [15]

Therefore, the development of a 0.01% atropine solution manufactured and marketed by Laboratorios Sophia, S.A. de C.V., would represent a great opportunity as a treatment to prevent progression in the face of the increase in myopia cases worldwide that has been recorded in recent years.

The Laboratory has experience in preclinical and clinical studies with products with 1% atropine, as well as experience in commercialization. However, having no experience with the formulation of PRO-201, it is important to develop the appropriate clinical research.

2.6 Rationale for the study

Laboratorios Sophia, S.A. de C.V., has carried out preclinical and phase I clinical studies of a similar formulation, but with a higher concentration of the component. The decrease in the concentration of the main component (atropine sulfate) in the PRO-201 solution to this formulation studied could mean a greater safety of PRO-201, not necessarily a greater tolerance, since the excipients may be involved with clinical manifestations unrelated to the main components.

Therefore, the present study serves to test the safety and tolerability of the PRO-201 product, 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 the PRO-201 formulation manufactured by Laboratorios Sophia S.A. de C.V. on the ocular surface of ophthalmologically and clinically healthy subjects.

3.1.2 Specific objectives:

3.1.2.1 Primary

- To evaluate the safety of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers, through the incidence of unexpected adverse events (AEs) related to the investigational product (PI).
- To assess the tolerability of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers using the incidence of photophobia.

3.1.2.2 Secondary

- To evaluate the safety of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers, by evaluating the pupillary diameter by the OPD scan.
- To evaluate the safety of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers, using the incidence of expected PI-related AEs.
- To evaluate the tolerability of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers, by evaluating the best corrected near visual acuity (CVA) with the ETDRS primer.

3.1.2.3 Exploratory

- To assess the safety of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers, by assessing best-corrected visual acuity (BCVA).
- To evaluate the safety of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers, by assessing intraocular pressure (IOP).
- To evaluate the safety of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers, by evaluating corneal and conjunctival staining with fluorescein and lysmine green.
- To assess the safety of PRO-201 ophthalmic solution applied to the ocular surface in healthy volunteers, by assessing vital signs (heart rate and blood pressure).
- To evaluate the tolerability of PRO-201 ophthalmic solution by means of the Ocular Comfort Index (OCI) score.

3.2 Hypothesis

H0= The PRO-201 ophthalmic solution is not safe in its ophthalmic application as it has an incidence of unexpectedly related adverse events (AEs) greater than 10% and is not tolerable as it presents an incidence of photophobia greater than 15% of the study population.

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

H1= PRO-201 ophthalmic solution is safe in ophthalmic application with an incidence of unexpected, related adverse events (AEs) of less than 10% and is tolerable with an incidence of photophobia of less than 15% of the study population.

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

4. Study design

4.1 Study Overview

Phase I Clinical Trial, Controlled, Non-Comparative, Open-label, Single-Center

4.2 Rationale for the study design

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

4.3 Expected duration

The total duration of the study, from the first visit of the first patient to the preparation of the final report, is estimated to be 6 months.

The planned recruitment period is 4 months. Considering that the proposed sample is 29 subjects, the total average recruitment rate during the study should be no less than 2 subjects included each week.

The approximate duration of each subject in the study is up to 22 days.

5. Study population

5.1 Eligibility Criteria

5.1.1 Inclusion criteria

- Be clinically healthy
- Can voluntarily give their signed informed consent (CRF)
- Be able and willing to comply with scheduled visits, treatment plan, and other study procedures
- Be between 18 and 35 years old.
- No history of contact lens use
- Women of childbearing potential must ensure continued use (initiated ≥ 30 days prior to signing the CRF) of hormonal contraceptive or intrauterine device (IUD) use during the study period
- Have a better far-corrected visual acuity of 20/30 (logMAR 0.2) or better in both eyes.
- Have a better corrected visual acuity close to 20/25 (logMAR 0.1) or better in both eyes.
- Have vital signs within normal parameters.
- Have an IOP ≥10 and ≤ 21 mmHg.

5.1.2 Exclusion criteria:

- Be a user of topical ophthalmic products of any kind.
- Allergy or known intolerance to ingredients in atropine eye drops and other derivatives of muscarinic antireceptor agents.
- Being a user of medicines, or herbal products (plant extracts, infusions, naturopathic preparations, homeopathics, etc.), by any other means of administration.
- For women: being pregnant, breastfeeding, or planning to become pregnant within the study period.
- Have participated in any investigational clinical study 90 days prior to inclusion in the present study.
- Have previously participated in this same study.
- Who are unable to follow the lifestyle considerations described in Annex 1.
- Have a history of any chronic-degenerative disease, including diabetes and high blood pressure.
- Have an active inflammatory or infectious disease at the time of study entry.
- Have unresolved injuries or trauma at the time of study entry.
- Have a history of any type of eye surgery.
- Having undergone surgical, non-ophthalmologic procedures within the past 3 months.
- Be or have an immediate family member (e.g., spouse, parent/legal guardian, sibling, or child) who is employed by the research site or sponsor, and who is directly involved in this study.
-

5.2 Criteria for elimination and/or substitution of subjects

5.2.1 Elimination Criteria

- Withdrawal of the letter from the FCI.
- Presentation of a serious adverse event related or not to the investigational product, which at the discretion of the PI and/or sponsor could affect the patient's ability to continue with the study procedures safely.
- No tolerability or hypersensitivity to any of the compounds used during the tests (fluorescein, lysmine green or tetracaine).
- No tolerability or hypersensitivity to any of the investigational drugs.
- Adherence < 90% determined by the subject's diary

5.2.2 Substitution of subjects

The sponsor, with the prior authorization of the research ethics committees, may decide to replace the subjects who withdraw their FCI or those who present loss of follow-up, until the necessary sample size is completed.

5.3 Counting failures

A screening failure is defined as those participants who agree to participate in the study, giving their consent, but who are not assigned to the treatment group, i.e., do not enter the study. It is necessary to report at least the following information on counting failures:

- Demographics.
- Details of the failure to count (specify whether it is due to eligibility criteria, which one, or some other reason for the failure).
- Presence of serious adverse events during the count.

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

5.4 Recruitment and retention strategies

The duration of the subject's participation in the study is approximately 21 days, during which time they must attend three visits in total and a safety call will be made, which corresponds to the baseline visits, visit 1, final visit and safety call. Strategies to improve subject retention include, but are not limited to:

- Clearly inform the objectives of the study.
- Make calls or send text messages to remind you of appointments or activities to do.
- Provide a printed calendar and an identification card to remember appointments and activities that will be carried out, in addition to the estimated time of their duration.

- Systematic organization of the study procedures, so that the subject does not last longer than necessary in 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 to the PI, who at some points were active subjects of the study, but their final evaluation could not be completed.

If the subject does not terminate their participation due to withdrawal of consent or major deviation, the last visit, in which their withdrawal was determined, will be considered their final visit. Subjects who are removed due to the presence of AEs will continue with the follow-up that is defined until the closure of their AE.

In cases where the participating subject does not attend their appointment, the research center must make a call to find out the reason and will try to make a new appointment within the established window period or an unscheduled appointment. If it is not possible to make an appointment, the subject will be considered as loss of follow-up and will be asked about the presence of adverse events and the reason for leaving the study, as minimum data.

5.6 Identification of the subject

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

The initials of the study subject 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 compound surname, the first letter will always be used.

Example:

A) Arieh Daniel Carrizalez Market
to. Initials: AMC

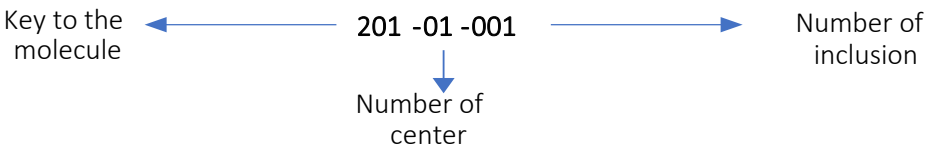
B) Juan De la Torre Orozco
b. Initials: JDO

In the counting stage, the participant number will be assigned consecutively, using 3 consecutive digits.

Once the subject has been selected, they will be assigned a number with which they will be identified throughout the study. This code will be made up of eight numbers in the following order from left to right:

- three digits of the molecule under study according to the name by the sponsor.
- Two digits correspond to the research center number.
- three digits of the number following their inclusion assigned in the research center.

Example of assigned number:



6. Investigational product and treatment

6.1 Managed Products

6.1.1 Investigational product

- PRO-201 . Atropine Sulfate 0.01% Ophthalmic solution. Zapopan, Jalisco, Mexico. Laboratorios Sophia, S.A. de C.V
- Route of administration: Ophthalmic.

6.1.2 Investigational Product Dosage

- Dosage: 1 drop once a day (QD) in the evening, in both eyes (AO).

6.1.3 Treatment with the investigational product

The investigational product will be delivered at the conclusion of the baseline visit, and the investigational subject will administer the drops at night, applying a total of one drop at night to each eye during the 14 days of application.

The baseline visits and visit 1 should be done in the morning shift, before 4:00 p.m., so that the patient can have enough time to apply PRO-201 at the indicated time.

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

The delivery will be made by means of a courier service contracted by the sponsor, expressly selected for this purpose, to the address of the research center according to the study plan.

The reception will be carried out by the assigned personnel of the research team. You will need to check that the primary packaging (box) is in good condition. If you show alterations or defects in its integrity that in your judgment could have damaged the content, you must report it to the sponsor. If the package does not show significant defects, it will proceed to open it.

Inside the shipment you must locate the acknowledgment document and the temperature meter (*data logger*). You must check that the temperature recorded complies with what is specified for transport and safekeeping. It will verify the content (PI) with what is reported in the document. In case the document corresponds to the content, you will sign the receipt and send it to the sponsor. Otherwise, it will notify the sponsor.

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

The storage temperature should be between 2° and 30°C.

From its receipt at the center and until there is no stock of PI stored, the research center has the obligation to review the storage conditions of the PI daily and manually record, in the designated

format, the temperature set by the *data logger* (current, minimum and maximum temperature). Such data will be reviewed by the clinical monitor during their monitoring visits according to the records stored in the *data logger's memory.*

In the event of loss of material, it must be documented in the logbook of inputs and outputs along with a clear description of the mechanism by which the loss occurred.

Upon completion of the protocol, all study material will be retrieved by the sponsor as part of the closing visit. The final delivery of materials will be made by the principal investigator or the person designated by him to deliver material 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 a lack of undocumented material at the conclusion of the study.

6.3 Concomitant treatments and medications (permitted and prohibited)

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

Allowed medications:

- Ophthalmic:

All permitted medications applied via ophthalmic medication during the study must wait a minimum period of 10 minutes from the last application of the treatments under study or reference. The above is to avoid the interaction of treatments in the tear film, based on the flow index and physiological tear volume. [26]

  - Tetracaine 0.5%
  - Tropicamide 0.8% /Phenylephrine 5%
  - Hypromellose 2%
  - Fluorescein
  - Lysmine Green

Prohibited Medications:

- Any ophthalmic medications that are not on the list of allowed medications
- Any systemic medication

6.4 Procedure for monitoring and measuring adherence

For more than four decades, there has been a lot of research on the appropriate way to measure and quantify medication adherence, but none has reached a consensus to stand as the gold standard, both in cross-sectional and longitudinal studies. [27, 28, 29, 30, 31, 32, 33, 34]

There are different procedures to measure adherence to pharmacological interventions. The most common procedure includes self-reports, which include patient interviews, questionnaires and self-monitoring diaries. Its strengths are speed, flexibility, low cost and ease of implementation; They have a high degree of specificity for non-adherence, however, the sensitivity and reliability for adherence is low. [34, 35]

The biochemical measurement of the drug, or its metabolite, is one of the methods that best confirms the use of the drug. However, in addition to raising costs and being impractical, it is of little use in the context of ophthalmic applications, since concentrations at the peripheral level could be undetectable; and samples of other tissues involve more invasive methods that would not be advisable. [34]

There is no standardized parameter to define adequate adherence, it must be defined and delineated by the objectives of the research. [34]

For this study, a minimum adherence of 90% (per subject's diary) will be necessary to meet the objectives of the research. Therefore, subjects with adherence of less than 90% will not be considered for the evaluation of exploratory variables but will be considered for the safety and tolerability variables in the intention-to-treat population group.

6.5 Strategies to improve adherence

1. The PI will sensitize the subject to the importance, to achieve the objectives of the study, of the correct application of the concomitant treatment.
2. Direct questioning by the PI about the application of the 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 CEI.

7. Study methods and procedures

7.1 Research center

This study will be carried out in a research center previously evaluated by the sponsor. The center will be an institution or establishment where health research is carried out that 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 their prerogative to design the organization and select the personnel who will perform the functions. However, it is necessary for the sponsor to sponsor that the PI and sub-investigator be ophthalmology specialists.

Any person who is designated, under the responsibility of the PI, as a part of the study monitoring (sub-investigator, nurse, etc.) or a specific function of participation in the study (pharmacist, administrative assistant, study coordinator, etc.) must appear in the "Delegation of Responsibilities".

The competence and training of any person who has direct participation in the activities of the study must be verified prior to the performance of any activity related to the protocol. The above must be recorded and the documents that constitute evidence of this competence and/or training must be kept in the master file of the study. The competence and training of the staff who have functions in the study, both at the central level and in the study center, is the responsibility of the sponsor.

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

7.2 Clinical Study Registration

This clinical study will be registered by the sponsor in public clinical trial registries before its start (inclusion of the first subject): National Registry of Clinical Trials (RNEC) of the Federal Commission for the Protection against Health Risks (COFEPRIS) and in a WHO primary registry platform. WHO Primary Registries meet specific criteria for 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 Assignment of treatment

- It is a non-comparative, open-label study. All patients will receive the same treatment.

7.4 Outcome variables

7.4.1 Primary outcome variables

- Incidence of unexpected PI-related AEs.
- Incidence of IP-related photophobia.

7.4.2 Secondary outcome variables

- Changes in pupillary diameter.
- Incidence of expected PI-related AEs.
- Changes in visual acuity.

7.4.3 Exploratory variables

- Changes in the AVMC.
- Intraocular pressure (IOP) changes.
- Corneal and conjunctival staining changes with fluorescein and lysmine green.
- Changes in vital signs (heart rate and blood pressure).
- Changes in OCI.

7.4.4 Definition of variables, methods and scales for their measurement

Board 1. Operational definition of variables

Variable	Conceptual Definition	Operational Definition	Measurement Type	Reference value	Statistical test
Incidence of unexpected-IP-related AEs	Any adverse medical event that occurs in the clinical research subject to whom the PI was administered that has a certain causal relationship with the PI and is not expected. [36]	All AEs (according to the protocol) in which the PI suspects a causal relationship with the PI will be reported.	• Discrete quantitative • Nominal qualitative	< 10%	• U for Mann Whitney • *Pearson X2* or Fisher Exact*
Photophobia incidence	Expected-PI-related AE	Present / Absent	• Discrete quantitative • Nominal qualitative	< 15%	• U for Mann Whitney • *Pearson X2* or Fisher Exact*
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 depends on the pupillary diameter, which in turn	The pupillary diameter results from the balance between the pupil sphincter muscle and the radial fibers of the iris that have only autonomic innervation. [37] It will be evaluated through the OPD scan.	Continuous quantitative	2 – 6 mm	Kolmogorov-Smirnov or Mann Whitney U*

Variable	Conceptual Definition	Operational Definition	Measurement Type	Reference value	Statistical test
	varies depending on the ambient lighting.				
Incidence of AD	Any adverse medical event that occurs in the clinical research subject to whom the PI was administered. [36]	All AEs (according to the protocol) will be reported and not just those where the PI suspects a causal relationship with the PI	• Discrete quantitative • Nominal qualitative	• Incidence • Seriousness (severity) • Causality	• U for Mann Whitney • *Pearson X2* or Fisher Exact*
Changes in the AVMCc	Clinical assessment of near visual acuity allows visual ability to distinguish letters to be assessed.	It will be evaluated by means of the ETDRS booklet for near vision (annex16.1 ETDRS Primer for Near Vision), using the font size in logMAR.	Continuous quantitative	0.1 or better	Kolmogorov-Smirnov or Mann Whitney U*
Changes in the AVMC	Visual spatial acuity is the ability to distinguish separate elements of an object and identify them. 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 Primer on logMAR	Continuous quantitative	0.2 or better	Kolmogorov-Smirnov or Mann Whitney U*
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	Kolmogorov-Smirnov or Mann Whitney U*
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 SICCA (see annex 16.2 SICCA Eye Staining Rating (modified from Whitcher et al, 2010) [38].	Discrete quantitative	0 to 12	Kolmogorov-Smirnov or Mann Whitney U*
Changes in vital signs	It includes the measurement of HR, SBP and DBP. They are clinical parameters that reflect the physiological state of the organism.	HR, SBP and DBP will be measured with an electronic blood pressure monitor or manually.	Discrete quantitative	HR: 60 – 100 bpm SBP: 120 – 139 mmHg DBT: 80 – 89 mmHg	Kolmogorov-Smirnov or Mann Whitney U*
OCI Score	The OCI is a questionnaire designed to measure irritation of the ocular surface, it assesses symptoms focused on the comfort associated with ocular surface alterations.	The evaluator will apply the questionnaire to the subject and will allow the subject to answer it calmly without any pressure and/or	Discrete quantitative	0 – 100	Kolmogorov-Smirnov or Mann Whitney U*

Variable	Conceptual Definition	Operational Definition	Measurement Type	Reference value	Statistical test
	Elevated values indicate more severe symptoms.	coercion (see annex 16.3 Ocular Comfort Index (OCI)			

Abbreviations: AVMC, best corrected visual acuity; CVA, near best corrected visual acuity; AE, adverse event; HR, heart rate; OCI, ocular comfort index; PI, principal investigator; bpm, beats per minute; DBP, diastolic blood pressure; SBP, systolic blood pressure; IOP, intraocular pressure; rpm, breaths per minute; TF, fluorescein staining; IP; principal investigator; X^2, Chi-square. *When applicable.

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

For clinical purposes, ophthalmological evaluations will be carried out in both eyes, and their record will be recorded in the clinical file. However, to obtain results and comply with the assumption of independence within the variables, only examinations and qualifications of the right eye will be recorded in the eCRF when appropriate. Next, the description of the variables is made, which are not in order of execution.

7.4.5.1 Intraocular pressure

Tonometry is the objective measurement of IOP, based primarily on the force required to flatten the cornea or the degree of corneal indentation produced by a fixed force. Goldman tonometry is based on the Imbert-Fick principle. [39]

The tonometry will be performed, after instillation of the topical anesthetic, with fluorescein and the use of the cobalt blue filter (after the evaluation of the surface staining). 2 samples will be taken, and the average will be calculated, which will be recorded in the clinical file. The average will be recorded in the eCRF.

Management as AE: IOP peaks ≥24mmHg should be reported as AEs.

7.4.5.2 Heart rate

Heart rate is the number of times the heart ventricles contract per unit of time, usually per minute. The heart rate measurement will be done by direct auscultation in the chest with a stethoscope. In the case of measuring, it in women, it may be allowed to measure it on the pulse of the wrist. However, the method chosen for the patient will be the same to be used on all visits. [40]

For the measurement of the heart rate, it will be necessary for the patient to be calm and in a state of rest. You will be asked to rest for at least 20 minutes upon arrival at the clinic, before your measurement, and when you are due to be assessed after the application of the medication, you should also have a 20-minute rest period.

For protocol issues, we refer to rest or rest as the state where the patient does not have agitation because of unnecessary physical effort. The patient should be seated for most of the rest of the period, being able to get up to walk to go to the bathroom.

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

7.4.5.3 Systemic Blood Pressure

It is the force exerted by blood circulation on the walls of the arteries. Blood pressure is assessed with two measurements: systolic (measured when the heart beats and is when the pressure is at the highest) and diastolic (measured between beats, when the pressure is at the lowest point). When it is reported, the systolic is written first and then the diastolic. [41]

A calibrated aerobic or mercury sphygmomanometer and stethoscope will be used to measure it. The left arm should be used for the measurement, and it should be done at rest.

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

7.4.5.4 Photophobia

Photophobia is a sensory disturbance caused by light, it is considered a common symptom seen in neurological and ophthalmological conditions. According to Albilali (2018) it can be induced by glare; It was described as "uncomfortable vision, based on the diffusion of light through the ocular means or on a transient or permanent lack of adaptation." It involves an uncomfortable sensation of excessive brightness without pain, and occurs mainly with eye diseases, such as toxic, amblyopia, albinism, rod monochromatism, and corneal, lenticular, and vitreous opacities. In this study, it may occur secondary to pharmacological mydriasis generated using atropine sulfate. [42]

Photophobia will be assessed within the adverse event interrogation according to the 8. Evaluation and management of adverse events, without questioning in a directed way within visit 1, final visit and security call. It will be reported as present or absent.

7.4.5.5 Pupillary diameter

It is the hole in the middle of the iris through which light penetrates the eyeball. Its diameter results from the balance between the pupil sphincter muscle and the radial fibers of the iris (pupil dilator). Normal values are 2-6 mm. [37]

In higher concentrations this product is mydriatic, however, due to the nature of the research and the physicochemical characteristics this reaction is not expected to occur, we will consider as significant the change that is generated in the pupil of the research subjects > 2 mm (pharmacological mydriasis), whether this change in photopic or mesopic vision. The method to perform the measurement will be using the following tool: OPD-Scan III (NIDEK CO., LTD, Tokyo, Japan). It is requested that the tool used be the same for all patients of the research center and for all their visits. It is necessary that the measurement is always done in the same office, with the same artificial light conditions and that the patient has at least 5 minutes inside the place before the measurement.

If there is a change greater than 2 mm between baseline and any of the other measurements, the product will be considered to have produced pharmacological mydriasis. This change will also be considered an adverse event.

7.4.5.6 Best-corrected visual acuity

Visual acuity (VA) is a test of visual function. Visual spatial acuity is the ability to distinguish separate elements of an object and identify them. It is quantified as the minimum angle of separation (located at the nodal point of the eye) between two objects that allow them to be perceived as separate objects.

Snellen's 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 arc minutes. 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 place (i.e., 20/20 = 1 and 20/40 = 0.5). [34]

VA will be evaluated at baseline, without refractive correction with the Snellen chart. It will be in a place with adequate lighting (natural or artificial) and at 3m from the subject to be evaluated. Visual acuity of the right eye will be taken initially, asking the subject to hold both eyes open and using an occlude to cover the contralateral eye; the subject will read aloud the lines that the evaluator points out, the line of smaller letters that he can see will be noted by the evaluator in fraction as the AV of OD in the clinical record and the eCRF, then the same evaluation will be performed in the left eye and its record only in the clinical file.

Next, the subject's best refractive correction (obtained by autokeratorefractometer and subjective tests) will be evaluated and the examination will be repeated using the refraction obtained. This result will be reported as best-corrected visual acuity, both data will be reported for statistical purposes in logarithmic figures (LogMAR) and in eCRF.

A fraction conversion table is attached to LogMAR. (see annex 16.4 Fraction to LogMAR conversion table.)

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

7.4.5.7 Best Corrected Visual Acuity Near

Visual Acuity (VA) is the characteristic of vision that determines the ability to detect, discriminate, and recognize objects on a background at a close distance. It assesses macular function and provides information on the accuracy of retinal focus, as well as the integrity of the neurological elements of the eye and the interpretive ability of the brain.

Near visual acuity (NVA) will be assessed, without refractive correction with the ETDRS chart. It will be in a place with adequate lighting (natural or artificial) and at 35 to 40 cm from the subject to be

evaluated. Visual acuity of the right eye will be taken initially, asking the subject to hold both eyes open and using an occluder to cover the contralateral eye; the subject will read aloud the lines that the evaluator points out, the line of smaller letters that he can see will be noted by the evaluator in fraction as the AVc of OD in the clinical record and the eCRF, then the same evaluation will be performed on the left eye and its record only in the clinical file.

Next, the subject's best refractive correction (obtained by autokeratorefractometer and subjective tests) will be evaluated and the examination will be repeated using the refraction obtained. This result will be reported as best corrected near visual acuity (CVA), both data will be reported for statistical purposes in logarithmic figures (LogMAR) and in the eCRF. It is necessary that the measurement is always done in the same office, with the same light conditions.

A fraction conversion table is attached to LogMAR. (see annex 16.4 Fraction to LogMAR conversion table.)

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

7.4.5.8 SICCA Ocular Surface Staining

The sequence of staining of the ocular surface is important for its reproduction and accuracy. The application of fluorescein should be done before the application of lysmine green.

Fluorescein (TF) staining: A drop of topical anesthetic will be instilled in the bottom of the conjunctival sac, then a second drop will be 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 is made with the conjunctiva at the fundus of the temporal sac, while the patient looks upwards, without damaging the conjunctiva. After evaluating the TRPL, between 4 and 8 minutes after the instillation of the stain, the evaluation of the fluorescein stain in the cornea is performed, with the slit lamp with a cobalt blue filter. It will be graded according to the Sjögren International Clinical Collaboration Alliance (SICCA) Eye Staining Rating (CTO). [43]

According to the CTO, grade 0 corresponds to the absence of punctate epithelial erosions (PES); grade 1 is defined as the presence of 1-5 EEP; grade 2 corresponds to 6-30 EEP; and >30 EEP will be classified as grade 3. Additionally, one rating point will be added if: 1) EEP is present in the central portion of the cornea with a diameter of 4mm; 2) filaments are observed and 3) confluent staining patches are observed, including linear stains. See Figure 2. [43]

The PI will record in the file and the eCFR the grade granted for the corneal staining of OD and OS, respectively. The maximum score per eye is 6.

Lysmine green (TVL) staining: After revision with fluorescein, a drop of saline solution will be applied to the tip of the lysmine green strip allowing it to sit on the strip for 5 seconds to elute the dye. A drop of the strip is instilled at the bottom of the temporal sac, while the patient looks upwards,

without damaging the conjunctiva. The patient may be asked to blink repeatedly to prevent accumulations in the conjunctival folds. The examination should be done between 1 and 4 minutes after installation through a neutral density filter or with the red-free filter. It will be graded according to the CTO of the SICCA. [43]

In CTO, grade 0 is defined as the presence of 0 to 9 spots of lysmine green staining in the interpalpebral bulbar conjunctiva (grading the temporal and nasal portion separately); grade 1 is defined by the presence of 10 to 32 points; grade 2 by 33 to 100; and grade 3 for >100 points. Due to the difficulty in counting individual points in a moving eye, any area ≥ 4mm^2 of confluent points is considered >100 points. See Figure 2. [43]

The PI will record, in the file and in the eCFR, the sum of the grades given to the temporal and nasal portion for the OD, then the same evaluation will be performed in the OI and its record only in the clinical file. The maximum score per eye is 6.

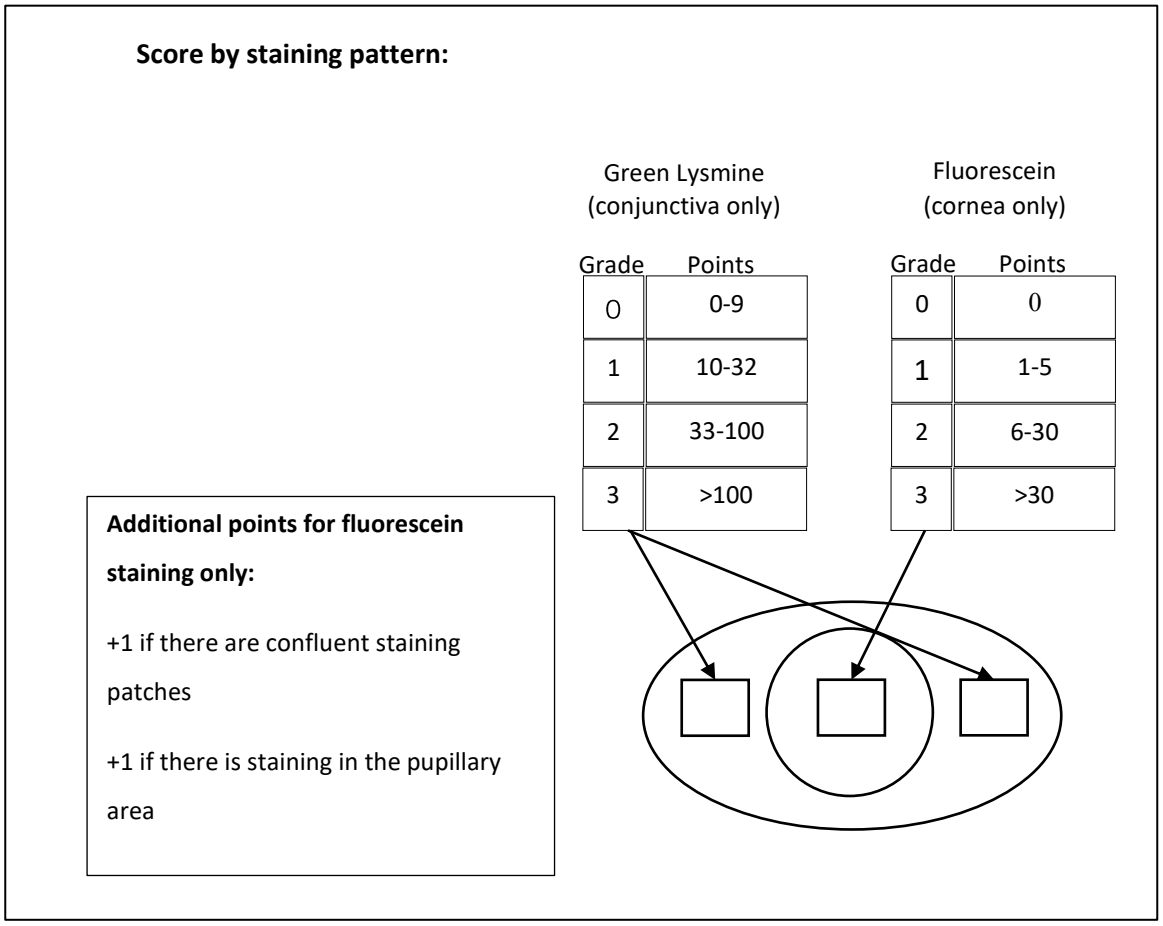


Figure 1. SICCA Eye Staining Rating (Modified from Whitcher et al, 2010) [38]

Ocular surface staining by SICCA assessment is determined by the sum of corneal fluorescein staining and conjunctival staining with lysmine green. As seen in Figure 2. The values that can be obtained for each eye are from 0 to 12, and abnormal staining is considered when the value is 3 or greater.

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

7.4.5.9 Adverse events

As described in the 8. Evaluation and management of adverse events An adverse event is defined as any unfavorable medical occurrence in a subject to whom an investigational product is administered, regardless of causal attribution.

Adverse event management shall be carried out as described in section 8. Evaluation and management of adverse events and incidents.

The Principal Investigator will record in the corresponding section of the eCRF the adverse events that the study subjects may present in addition to referring them in the clinical record.

For an adequate evaluation of adverse events, in addition to directed questioning, it is necessary to perform a comprehensive ophthalmological evaluation at each visit, which consists of ophthalmological examination of the eyelids and adnexa; anterior and posterior segment that is performed in a routine ophthalmological check-up, whose procedures are not specifically included in the study variables. Posterior pole evaluation can be with direct or indirect ophthalmoscopy, with or without pharmacological mydriasis, at the discretion of the PI. An assessment of the fundus will be carried out in search of abnormalities that alter the result of the study. IOP will be measured in this evaluation, with the PI choice instrument, it should be measured after the evaluation of stains. The result of the assessment will be recorded in the clinical file. In the eCRF, only findings that are considered so by the Principal Investigator will be reported as an adverse event.

The adverse events that can occur with PRO-201 according to what is reported in the literature are: photophobia, mydriasis, difficulty reading, ocular allergic reactions (atopic dermatitis, allergic conjunctivitis and other allergic reactions), eye irritation, eye burning, eye pain, tearing, foreign body sensation, dry eye, hyperemia and/or red eye, blurred vision, eye itching; Systemic adverse events include: headache, drowsiness, dizziness, tachycardia, flushing, restlessness, irritability, dry skin, dry mouth, skin rash and decreased state of alert. [8] [6] [18] [19] [20] [21] [22] [23] [24]

7.4.5.10 Respiratory rate

It is the measurement of the breaths per minute that the subjects have per research. It is discreetly evaluated by observing, without the subject being aware of it, the movements of the rib cage, counting as one breath each increase/elevation of the subject's rib cage. 12 to 24 breaths per minute is considered normal.

Management as AE: Any value outside of the normal will be considered an adverse event. It is possible that in some cases the research subject should be considered as a possible case of a patient with COVID-19, and the investigator should refer the patient to medical attention and withdraw the subject from the investigation.

7.4.5.11 Body temperature

It is the degree or thermal level of a body. Normal values range from 35°C to 37.4°C. It can be taken by a contact thermometer or not, in different parts of the body. In the study, any thermometer calibrated at the research center may be used, if the same one is used for all patients and for all their visits.

Management as AE: Any value outside of the normal will be considered an adverse event. It is possible that in some cases the research subject should be considered as a possible case of a patient with COVID-19, and the investigator should refer the patient to medical attention and withdraw the subject from the investigation.

7.4.5.12 Ocular comfort index

It is a questionnaire designed to measure ocular surface irritation with Rasch analysis to produce estimates on a linear interval scale (ratings: 0-100). Like the index for ocular surface diseases, the ocular comfort index (OCI) assesses symptoms. The OCI contains 8 items (one positive and eight negative) that focus on discomfort associated with ocular surface alterations. Each of these questions has two parts, which separately inquire about the frequency and severity of symptoms. [44]See annex 16.3 Ocular Comfort Index (OCI)

At the baseline visit and final visit, the evaluator will deliver the questionnaire to the subject and allow the subject to answer it calmly without any pressure and/or coercion, only assisting him if he has difficulty understanding any of the questions.

7.5 Description of procedures or assessments during the study

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

7.5.1 Signing of informed consent

Procedure by which it is guaranteed that the subject under investigation has voluntarily expressed his intention to participate in this research, after having understood the information that has been given to him about the objectives of the research, benefits, discomforts, and possible risks.

7.5.2 Preparation of medical history (includes ophthalmological and general medical history)

It must be carried out in accordance with the provisions of NOM-004-SSA3-2012 of the clinical file. Considering that the clinical record is the unique set of information and personal data of a patient, which is integrated into any type of establishment for medical care, whether public, social or private, which consists of written, graphic, imaging, electronic, magnetic, electromagnetic, optical, magneto-optical and any other type of documents, in which, Health personnel must make the records, annotations, where appropriate, certificates and certifications corresponding to their intervention in the patient's medical care, in accordance with the applicable legal provisions. [45]

And it will consist of:

- Medical history: It must be prepared by medical personnel and other professionals in the health area, in accordance with the specific information needs of each of them, it must have, in the order indicated, the following sections:
  - Interrogation. - It must have at least: identification form, if applicable, ethnic group, hereditary-family history, pathological and non-pathological personal history, current condition (inquire about previous conventional, alternative and traditional treatments) and interrogation by devices and systems (especially ophthalmological).
  - Physical exam. - You must have at least: vital signs (temperature, blood pressure, heart and respiratory rate), weight and height (when applicable).
  - Previous and current results of laboratory, cabinet and other studies (when applicable).
  - Diagnoses or clinical problems (when applicable).
  - Prognosis (when applicable).
  - Therapeutic indication (when applicable).

7.5.3 Eligibility Criteria

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

7.5.4 Assigning Subject Code

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 the 8. Evaluation and management of adverse events

7.5.6 Vital Signs Measurement

It is the measurement of heart rate, respiratory rate, blood pressure, and body temperature. These measurements can be made with a stethoscope, sphygmomanometer and mercury or digital thermometer.

Vital signs are described in the following sections: 7.4.5.2 Heart rate,7.4.5.3 Systemic Blood Pressure, 7.4.5.10 Respiratory rate and 7.4.5.11 Body temperature

7.5.7 Urine Pregnancy Test

The pregnancy test will be performed at the baseline visit and at the final visit. The Researcher must provide the research subject with a female, of reproductive age (there is no natural or induced menopause, defined as 12 consecutive months of amenorrhea). To perform the test, the patient will be allowed to go to the bathroom, have privacy for the performance of the test, and after the test is performed, the Investigator must corroborate the result by observing the medical device. [46]

7.5.8 Ophthalmological evaluation

Evaluation of your eyeball, eyelids, eyelashes, and other structures of your eyes by inspection, slit lamp (biomicroscopy), as well as palpation (touch). As part of this evaluation, visual acuity is taken with better corrected visual acuity, the integrity of the ocular surface is assessed, the anterior segment, intraocular pressure is taken, gonioscopy is assessed, and posterior segment (fundoscopy) is assessed.

7.5.8.1 Best-corrected visual acuity

Described in the 7.4.5.6 Best-corrected visual acuity

7.5.8.2 Best Corrected Visual Acuity Near

Described in the 7.4.5.7 Best Corrected Visual Acuity Near

7.5.8.3 Ocular Surface Integrity

This will be done by means of biomicroscopy using the slit lamp of the research center. An inspection of the cornea, conjunctiva, tear film, and eye stains with lysmine green and fluorescein will be carried out (in the section 7.4.5.8 SICCA Ocular Surface Staining stains are described.)

7.5.8.4 Previous Segment

It is the evaluation of the structures of the anterior segment [cornea, conjunctiva, anterior chamber, iris, pupil (includes measurement of pupillary diameter), lens, aqueous humor]. The evaluation will be made by means of the slit lamp.

7.5.8.5 Intraocular pressure

Described in the 7.4.5.1 Intraocular pressure

7.5.8.6 Gonioscopy

It is an 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 high viscosity solution (2% hypromellose) to improve the coupling of it with your cornea.

For the classification of the angle, the Shaffer system will be used.

Board 2. Shaffer's classification

DEGREE	ANGULAR OPENING	DESCRIPTION	RISK OF OCCLUDING
4	45°-35°	Open	Impossible
3	35°-20°	Open	Impossible
2	20°	Narrow	Possible
1	≤ 10°	Extremely narrow	Likely
0	0°	Closed	Occluded

Angular closure or suspected angular closure shall be Grade 2 or less in more than 180° of the angular circumferences.

7.5.8.7 Posterior Segment (Fundoscopy)

Also called ophthalmoscopy. This is the test done with a light and a magnifying glass to look at the fundus (optic nerve and retina) through the pupil. Sometimes it will be necessary to dilate the pupil so that the fundus of the eye can be evaluated much better.

7.5.9 Application of Medications During Visits

During patient visits, different 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 the measurement of intraocular pressure, application of dyes for staining and placement of the lens for gonioscopy.
- Ophthalmic solution of tropicamide 0.8% and phenylephrine 5%, used to dilate the pupil and better assess the posterior segment.
- Hypromellose ophthalmic solution 2%, may be used by the Investigator for the assessment of gonioscopy.

7.5.10 Delivery of material for the subject

It refers to the delivery of the subject's identification card and the subject's diary. The ID card will serve as an ID with the treatment assignment number; this card may serve as an appointment card. The Subject's Diary serves to keep track of the times the treatment is applied.

7.5.11 Delivery of Study Medication

Once the patient is in the study, the investigational product will be given, and the dosage of the product will be explained (one drop once a day at night in both eyes).

7.5.12 Evaluation of concomitant medicinal products

It is the question about the use of medications that they are taking regularly or that they have used in the last month. If you have required injected eye drug therapy, you should ask about the injected drugs in the last 6 months.

7.5.13 Assessment of adherence to treatment

It refers to indirectly evaluating the number of applications during the period between visits. To assess the approximate number of drops, the Subject's Journal can be reviewed to find out the applications registered.

7.5.14 Return of Study Medication

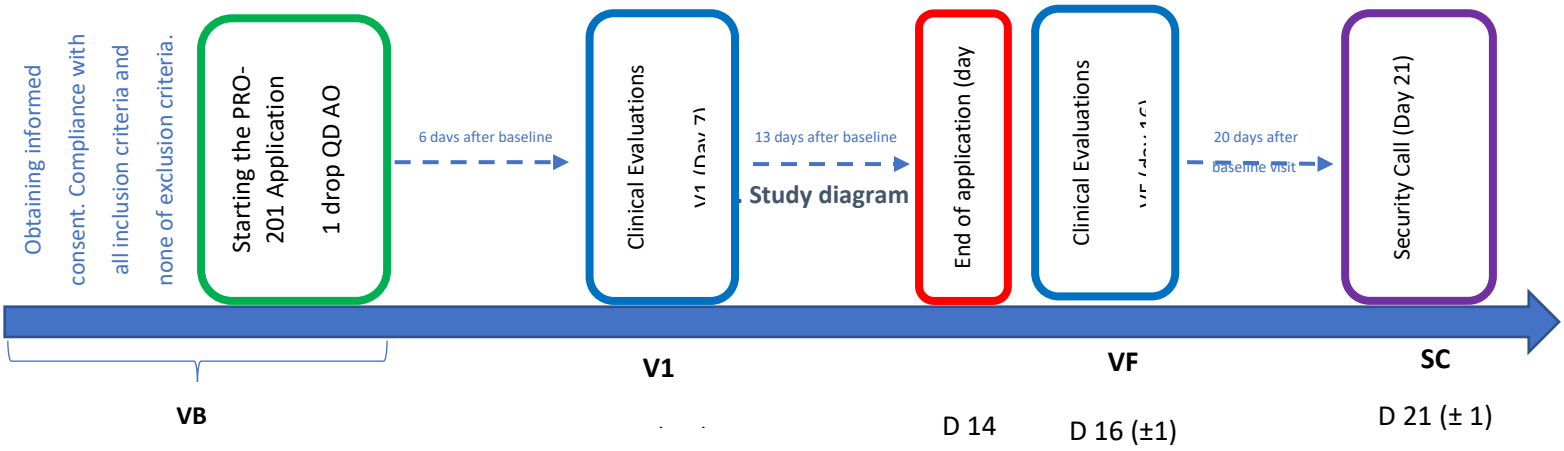
It refers to the return made by the research subject of the product under investigation.

7.5.15 Subject Journal Removal

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

7.6 Study Diagram and Timeline

7.6.1 Study diagram



BV= Basal visit

VF= Final visit. Evaluation of outcome variables

V1 = Visit 1

SC = Safety Call

QD= Once a day

AO= Both eyes

7.6.2 Study timeline

Board 3. Study timeline

Procedures	VB D 1	V1 D 7 ± 1	VF D 16 ± 1	LLS D 21 ± 1
FCI Signature	X			
Medical history	X			
Eligibility Criteria	X			
Concomitant Drug Evaluation	X	X	X	
Urine pregnancy test	X		X	
Vitals	X	X	X	
Best-corrected visual acuity	X	X	X	
Near Best Corrected Visual Acuity	X	X	X	
Pupillary diameter measurement	X	X	X	
Evaluation of fluorescein staining and lysmine green	X	X	X	
PIO	X	X	X	
Comprehensive ophthalmological evaluation	X	X	X	
Pharmacologic mydriasis (phenylephrine/tropicamide)	X			
Anterior chamber angle assessment (gonioscopy)	X			
Evaluating AEs	X	X	X	X
Subject and IP code assignment	X			
Eye comfort index	X		X	
Delivery of the PI and initiation of intervention	X			
Daily subject delivery	X			
Adherence assessment		X	X	
Return / evaluation of the subject's diary			X	
Return of PI			X	

Abbreviations: D, day; AEs, adverse events; FCI, informed consent form; LIS, security call; PI, research product; IOP, intraocular pressure; V, visit.

7.7 Procedures to be carried out per visit

Listed below are the procedures that will be performed at each visit, which may not show the optimal order for the Research Center. The investigator should order it according to his or her needs, and the needs of the study and sponsor.

The measurement of the pupillary diameter should always be done prior to pharmacological mydriasis in the case of the baseline visit and prior to the ophthalmological check-up at all visits. In the evaluation of adherence to treatment, it can be reviewed in the subject's diary.

7.7.1 Baseline visit

- Signing of informed consent
- Preparation of general and ophthalmological medical history
- Measurement of vital signs (temperature, blood pressure, heart rate, respiratory rate)
- Concomitant Drug Evaluation
- Pregnancy test (where applicable)
- Best-corrected visual acuity measurement
- Best Corrected Visual Acuity Measurement Near
- Pupillary Diameter Assessment
- Ophthalmological evaluation
- Fundus evaluation under pharmacologic mydriasis
- Evaluating the angle in the anterior chamber
- Fluorescein and Lysmine Green Eye Staining
- Intraocular pressure measurement
- Evaluation of adverse events
- Ocular Comfort Index (OCI)
- Subject Code Assignment and Research Product Delivery
- Submission of the subject's diary

7.7.2 Visit 1

- Measurement of vital signs (temperature, blood pressure, heart rate, respiratory rate)
- Concomitant Drug Evaluation
- Best-corrected visual acuity measurement
- Best Corrected Visual Acuity Measurement Near
- Ophthalmological evaluation
- Pupillary Diameter Assessment
- Fluorescein and Lysmine Green Eye Staining
- Intraocular pressure measurement
- Evaluation of adverse events
- Adherence Assessment

7.7.3 Final Visit

- Measurement of vital signs (temperature, blood pressure, heart rate, respiratory rate)
- Concomitant Drug Evaluation
- Pregnancy test (where applicable)
- Best-corrected visual acuity measurement
- Best Corrected Visual Acuity Measurement Near
- Ophthalmological evaluation
- Pupillary Diameter Assessment
- Fluorescein and Lysmine Green Eye Staining
- Intraocular pressure measurement
- Evaluation of adverse events
- Ocular Comfort Index (OCI)
- Adherence Assessment
- Return of the research product
- Return of subject's diary

7.7.4 Security Call

- Evaluation of adverse events

7.7.5 Unscheduled follow-up visits

At the request of the subject or personnel related to the study, unscheduled follow-up visits may be carried out for the reporting of adverse events or any situation that warrants it. During these visits, all pertinent data on the adverse events reported should be collected and, where appropriate, an appropriate management plan should be established.

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 analysis equipment) where the information is recorded for the first time, and which is part of the permanent records of the subject's history. Examples of source documents are medical history, clinical evolution notes, laboratory reports, cabinet study reports, nursing notes, follow-up notes, surgery records, etc.

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

7.8.2 Electronic forms of data collection

All data related to the protocol will be captured through an electronic case report form (eCRF) by the investigation team staff. The data related to the protocol should NOT be captured directly in the eCRF but should be transcribed from the corresponding source document. This procedure allows monitoring to verify the information captured in the eCRFs. It is the responsibility of the researcher to ensure that the information is transcribed into the eCRFs in a correct, complete, and timely manner. It is understood that all data captured and sent by the eCRF to data analysis is approved by the Researcher.

7.8.3 Archiving

The data collected in this database is anonymous (it only stores the subject number together with other information of interest). The program used for data capture and storage covers the traceability requirements necessary for the execution of clinical studies. The data collected will be stored by the sponsor or the clinical research organization designated for this purpose and its storage will have a duration of 10 years. The records of assignments of subject number will remain in the participating institutions in charge of the PI or his work team and must be kept for at least 5 years.

8. Evaluation and management of adverse events

8.1 Regulation and regulations 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 guidelines ICH E6. [36][47][48][49]

8.2 Definition of Adverse Event

According to the International Council on Harmonization (ICH), an adverse event (AE) is any unfavorable medical appearance in a clinical investigational subject who is administered a pharmaceutical product, regardless of causal attribution. [47] [48] [49]

Therefore, an AE can be any of the following: any unfavorable and unintentional disease, symptom, or sign (including an abnormal laboratory finding) that is temporally related to the use of a medical product, whether or not it is considered related to such a product; any new illness 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 symptoms or that results in a change in study treatment or concomitant treatment or discontinuation of study medication. [47] [48] [49]

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 be applied as any unwanted event that occurs during a clinical trial, including behavioral disorders. [49]

8.3 Use of adverse events as a study safety variable

Measuring the safety of the use of PRO-201 is paramount to the study, therefore, it is considered important to report any unwanted manifestations or diseases that occur during the study, regardless of whether or not the manifestation is considered to be related to the treatment under investigation. [49]

8.4 Definitions relevant to the classification of adverse events

Severity (serious/non-serious), also called seriousness (serious/non-serious). Serious or serious is defined as any event that: results in death, threatens life, requires hospitalization or prolongs hospitalization, is a cause of permanent or significant disability or disability, is the cause of alterations or malformations in the newborn, other medically important conditions.

Severity (mild, moderate, or severe). Mild is those that present with minimal symptoms, do not require treatment or suspension of the medication; moderate, when they interfere with usual

activities, without threatening the subject's life, require treatment and may or may not require discontinuation of the medication; severe, those that interfere with usual activities and require pharmacological treatment and discontinuation of the medication. [36][47][48]

Causality. It is the relationship that is 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 reappears with the administration of the pharmaceutical product; probably caused by the pharmaceutical product, there is a high suspicion of causality but no direct evidence is available or it is considered unnecessary or dangerous, i.e. the reaction disappears when the pharmaceutical product is discontinued; possibly caused by the pharmaceutical, there is additional information to suggest that the cause may be due to another pharmaceuticals or disease; unlikely to be caused by the pharmaceutical product, there is a clear explanation of the origin due to the underlying disease or the use of another pharmaceutical product; conditional, there is a lack of data to issue a clear causality; non-classifiable, those for which once all possible information has been obtained about the adverse event, it remains unclassifiable. [36] [47] [48] [49]

8.5 Responsibilities of the researcher

It is the responsibility of the Investigator to verify AEs through questioning, pertinent physical examination, assessment of evolution, as well as appropriate medical and pharmacological management; as well as to follow up until the resolution or outcome and definitive discharge of the AE, following the definitions determined in national and international regulations. [36] [47] [48]

In the event of an AE or any event that puts the health and well-being of the subjects at risk, pertinent medical care will be provided, either at the research center or will be referred to the Hospital Center with the highest resolution power with which the research center has a medical care agreement. The PI will notify the sponsor's clinical monitor, according to the times established in national and international regulations. In the case of serious adverse events, it will notify the sponsor and record the corresponding information in the eCRF and in turn, inform the IRB and IC.

The attention of the AEs will be carried out according to the event care diagram (see Figure 4. Adverse Event Care).

The sponsor's final report will include the report of adverse events in compliance with current national and international regulations. [36] [47]

If the research subject debuts during their participation in the study with any chronic adverse event, such as diabetes or systemic arterial hypertension, they will be referred to the competent health professional for chronic treatment. The follow-up and termination of your participation will be in accordance with the stipulations of the ICH.

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, gender, and if applicable specify the eye.
- Information about the causality of the adverse event, its relationship to investigational products, or to another drug related to the study, as appropriate.
- Important date information:
    - Date on which the adverse event occurs.
    - Date on which the Principal Investigator becomes aware of it.
    - Date of resolution or outcome, as applicable.
- Information on diagnosis and clinical management.
- Record the outcome or resolution of the event:
    - Recovered/resolved without sequelae
    - Recovered/resolved with sequelae
    - Not Recovered/Unresolved
    - Subject who presented death due to the adverse event
    - Subject who presented death and it is judged that the research product may have contributed
    - Subject who presented death and this is not related to the product under investigation
    - Unknown
- Information about the investigational product or product associated with the Adverse Event, Incident, Adverse Incident, AMR or SRAM must be recorded. The information essential for registration is the generic name, distinctive name or code of the investigational product or of the product associated with the undesirable clinical manifestation; it will also be necessary to enter the data concerning the batch number, manufacturing laboratory, expiration date, dose, route of administration, start and end dates of administration and/or consumption, reason for prescription; according to whether it is an investigational product or drug (protocol in which the subject currently participates) or whether it is a drug that the research subject consumes for the treatment of underlying concomitant diseases or uses for the management of some transitory sign or symptom that does not correspond to the Natural History of the pathology that motivated its entry into the research protocol.
- Indicate whether the removal of the suspected product (of causing the event) eliminates the adverse event. Also indicate if a dose adjustment is made, if the event changes in terms of intensity or seriousness, persistence of the reaction. It is important to indicate whether in those subjects who are exposed again to the product, which had previously been suspended, the AD reappears.
- Information regarding concomitant pharmacotherapy. Indicate the generic name, dose, route of administration, start and end dates of use, as well as the reason for the prescription, regardless of whether it is in accordance with the prescribing information or technical data

sheet or is used outside the regulations or what has been authorized by the local, national or international regulatory entity.

- Relevant medical history information. The analysis of the AE considers the information previously narrated, despite the clinical context in which this harmful phenomenon occurs in the participants of the clinical research protocol, is of special interest, so the information about previous conditions, hypersensitivity or allergy phenomena, previous surgical procedures, laboratory analyses or cabinet examinations that have been performed on the participant, etc., that the researcher deems it appropriate to mention may do so.

8.5.2 Adverse Event Tracking

The Principal Investigator will provide care and follow-up of the adverse event presented by the participant until its outcome, according to what is referred to in the following section.

8.5.2.1 Diagnosis against signs or symptoms

Whenever possible, an AE should be assessed/reported as a diagnosis and not as a sign or symptom (e.g., liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). If a diagnosis cannot be made from the signs or symptoms, then each sign or symptom should be recorded as an AE. If a diagnosis is made later, all AEs reported as signs or symptoms should be nullified and replaced by a single adverse event based on the diagnosis, with an onset date that corresponds to the onset date of the first symptom or sign of the eventual diagnosis.

8.5.2.2 Adverse events secondary to other adverse events

In general, AEs secondary to other AEs (cascading events or sequelae) should be identified according to the primary cause, with the exception of serious events. A medically important AE secondary to an AE that is separated in time from the initial event should be reported as a separate event in the EA section of the eCRF. For example:

If vomiting results in mild dehydration without additional treatment in a normal adult, vomiting should only be reported on the eCRF.

If vomiting results in severe dehydration, both events should be reported separately on the eCRF.

If severe gastrointestinal bleeding leads to renal failure, both events should be reported separately on the eCRF.

If a dizziness event leads to a fall and subsequent fracture, then all three events should be reported separately in the eCRF.

If neutropenia is accompanied by infection, both events should be captured independently in the eCRF.

All EAs must register separately if there are concerns regarding the association of events.

8.5.2.3 Persistent or recurrent adverse events

A persistent adverse event is one that spreads continuously without resolution between different patient assessment points. Such events only need to be registered once in the eCRF. The initial severity (intensity or degree) of the event will be recorded at the time of the first AD record. If a persistent AE becomes acute, the maximum severity should be recorded in the appropriate section of the eCRF. If the event becomes a serious event, it must be reported no later than 24 hours after the knowledge of the change in the status of the event. The eCRF section should be updated to reflect serious status and will record the date on which the event became serious, thus completing all relevant serious EA reporting data.

A recurrent AE is one that resolves between assessment points and subsequently reappears. Each recurrence of the EA must be recorded separately in the eCRF.

8.5.2.4 Abnormal vital signs

Not all abnormal vital signs qualify as AE. For an abnormal vital sign to be reported as an AD, it must meet any of the following criteria:

Be accompanied by clinical symptoms. It results in a change in treatment (dose modification, treatment interruption, etc.).

The report on abnormal values should focus on obtaining a diagnosis and not just a description of the abnormality.

8.5.2.5 Death

Death should be considered an outcome and not an event. The event or condition that caused or contributed to the fatal outcome should be recorded as the EA in the eCRF section.

8.5.2.6 Pre-existing medical conditions

A pre-existing medical condition should be recorded as AE only if the frequency, severity, or characteristics of the condition worsen during the study.

8.5.2.7 Hospitalization or prolonged hospitalization

Any AE that results in hospitalization or prolongation of hospitalization must be documented or reported as a serious AE with the following exceptions:

Hospitalization for pre-existing conditions if the following are met:

Hospitalization was planned before the study.

8.5.2.8 Pregnancies, miscarriages, and birth defects

Fertile women should contact their doctor to report any suspected pregnancy during the study. A pregnancy report must be issued, and the sponsor must be informed immediately.

Monitoring of the patient should continue until the outcome of the pregnancy. Pregnancy is not in itself an AE.

Any abortion should be classified as a serious AE.

Any birth defect or birth defect in a product from a woman who received the study drug should be classified as a serious AE.

8.5.3 Procedures for a serious adverse event

The process of attention to the adverse event considers the following stages:

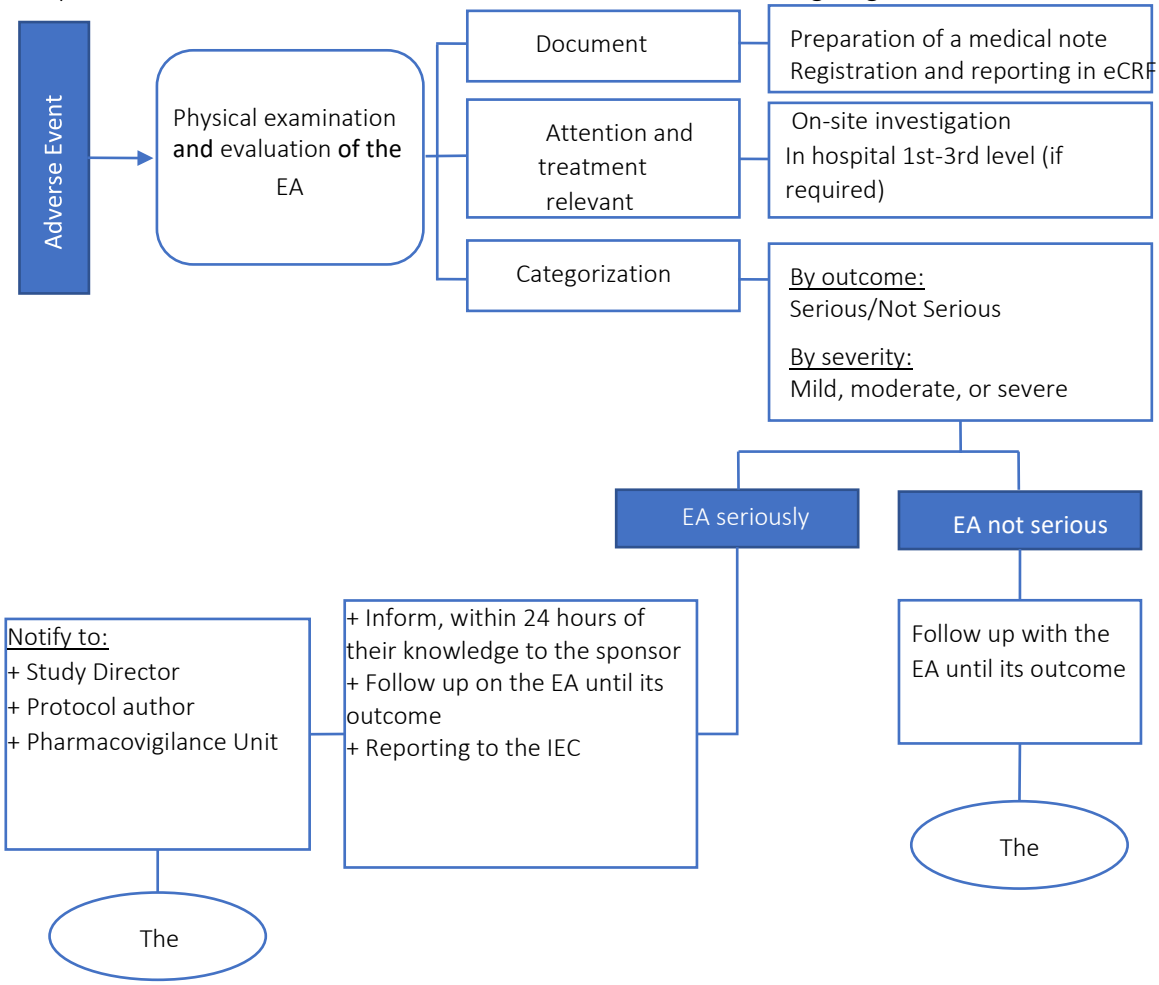


Figure 3. Adverse Event Care

During the development and conduct of this study, undesirable harmful events or adverse reactions/incidents, of medical implication, may occur in the research subject, which do not necessarily have a causal relationship with the investigational products. These harmful phenomena can occur during the use of investigational pharmaceutical products at doses authorized for use in humans by a local, national or international regulatory entity. However, it may be suspected that the

investigational product causes some unwanted clinical manifestation. AEs, Incidents, Adverse Incidents, ADRs or SRAMs to one or more pharmaceutical products can occur during the systematic evaluation of the participants (on the days when the clinical review is scheduled, according to the schedule of activities) or suddenly, in such a way that:

1. The investigator must be the first person to whom the subject notifies that he or she has developed or presented any harmful phenomenon of a clinical nature during his or her participation in this study.
2. According to his clinical judgment, Based on the pertinent physical examination, interrogation, etc., as well as the analysis of the information available in the medical literature and what is referred to in the Investigator's Manual, Information to prescribe or Summary of the Comparator Drug Label, the principal investigator determines the pertinent care of the harmful event/reaction.
3. Such care can be in the research center or in the hospital with the greatest resolution power. In such a way that, if the subject is sent by the PI to a hospital, he or she attends through a referral system. The reference can be with a card that identifies the subject as a study participant and links him or her to the pre-established agreement with the institution, or through a medical reference note issued by the Principal Investigator. Laboratorios Sophia, S.A. de C.V., will pay the expenses for the medical care of the participating subject, when the adverse event is associated with or is related to the product under investigation.
4. Taking the clinical information collected, either during the care provided at the research center or that provided by the treating physician(s) in the hospital, the PI will record the AE in its clinical note, stating the seriousness, intensity (mild, moderate or severe) and relationship with the investigational product.
5. The PI must migrate the relevant data to the eCRF and its respective adverse event section. By, in cases of serious adverse events, the clinical monitor of the study must be notified within 24 hours after learning it, so that in turn it informs the Clinical Team and the UTFLS, and that the CEI/CI is subsequently notified. Non-serious adverse events will be recorded and treated appropriately, and the safety profile of the investigational drug or PI will be reported to the appropriate regulatory entity in the final report of the clinical trial.

The recording of the outcome of the AE depends substantially on the follow-up that the Principal Investigator performs on the subject, since it is expected that most of the harmful phenomena (see subsection 2.5 Risk-benefit assessment and consult the Investigator's Manual) are ophthalmic in nature, however, there may be systemic alterations. Therefore, in the opinion of the researcher, the withdrawal of the participant or their permanence will be considered.

8.5.4 Causation assessment

Causality assessment is the methodology used to estimate the probability of attributing the observed adverse event to a pharmaceutical product. It considers probabilistic categories according to the available evidence and the quality of the information, based on the national pharmacovigilance and technovigilance regulations. [36][50]

An adverse event may or may not be related to the clinical trial. A causal relationship means that the intervention caused (or is reasonably likely to have caused) the adverse event. This usually implies a relationship between the time of the intervention and the adverse event (e.g., the adverse event occurred shortly after the subject under investigation received the intervention). [49]

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 the intervention, whether it is related to the experimental treatment, concomitant, surgical procedure, or diagnostic procedures performed during the study. [49]

Accepting that the adverse event is related to the clinical study requires a plausible mechanism of action—that is, that there is a logical sequence between the event that occurred and the intervention that caused it. In some cases, it is helpful to know the opinion of other doctors directly or indirectly involved in the research; as well as whether the patient considers that there is a relationship. [49]

The Pharmacovigilance and Technovigilance Unit of Sophia Laboratories (UFTLS) may use the categories of causality described by *the Uppsala Monitoring Centre*, to categorize the likelihood of adverse event to the investigational product or concomitant treatments or treatments used during visits: [36] [50]

- Definitive (certain): a clinical event, including alterations in laboratory tests, that manifests itself with a plausible temporal sequence in relation to the administration of the drug, and that cannot be explained by concurrent disease, or by other drugs or substances. The response to drug withdrawal (withdrawal) must be clinically plausible. The event must be definitive from a pharmacological or phenomenological point of view, using, if necessary, a conclusive re-exposure (challenge) procedure. [36] [50]
- Probable: A clinical event, including alterations in laboratory tests, that manifests itself with a reasonable time sequence in relation to the administration of the drug, that is unlikely to be attributed to concurrent disease, or to other drugs or substances, and that a clinically reasonable response is presented upon withdrawal. It is not necessary to have re-exposure (challenge) information to assign this definition [36] [50]
- Possible: A clinical event, including alterations in laboratory tests, that manifests itself with a reasonable time sequence in relation to the administration of the medicinal product, but which can also be explained by concurrent disease, or by other drugs or substances. Information regarding the withdrawal of the drug may be missing or unclear. [36] [50]

- Unlikely: A clinical event, including alterations in laboratory tests, that manifests itself with an unlikely temporal sequence in relation to the administration of the drug, and that can be more plausibly explained by concurrent disease, or by other drugs or substances. [36] [50]
- Conditional/Unclassified: A clinical event, including alterations in laboratory tests, reported as an adverse reaction, for which further data are essential for appropriate evaluation, or additional data are under review. [36] [50]
- Non-assessable/Unclassifiable: A notification that suggests an adverse reaction but cannot be judged because the information is insufficient or contradictory, and that cannot be verified or completed in its data. [36] [50]

Thus, the degree of certainty to establish the investigational product as the causal agent of the harmful phenomenon that occurs to the subject of the clinical study, can be indicated directly by the Principal Investigator based on his clinical experience or by applying the categories of causality described by *the Uppsala Monitoring Centre*. It is important that the researcher and the UFTLS consider 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, i.e. the presence of a common characteristic or pattern.

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

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, e.g., the appearance of abnormal metabolites or high levels of the drug or its biotransformation product.

f) Analogy, which refers to the experience gained 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. [51]

8.6 Unanticipated Issues

Unanticipated problems (PNA) consider those situations that pose risks to the participating subjects, in general, any incident, experience or result that meets all the following criteria:

- Unexpected in terms of its nature, severity, or frequency in relation to: 1) study-related documents such as investigator's manual, study protocol, and informed consent form; and 2) the characteristics of the population being studied.
- Related or possibly related to your participation in the study (possibly related means that there is a reasonable possibility that the incident, or results, was caused by study procedures).

- Indicative that the research places participants at a higher risk of harm (including physical, psychological, economic, or social) than previously recognized.

8.6.1 Reporting Unanticipated Issues

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

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

NAPs that are SEA must be reported to the IEC/IC and the sponsor within the first 24 hours of the PI becoming aware of it.

Any other NAPs will be reported to the IEC/IC and the sponsor within the first 5 business days after the PI 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, monitoring by study site, detection monitoring, reporting and tracking of adverse events, monitoring for resolution of discrepancies in data capture, 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 Monitoring the Study Site

The research center participating in the study will be monitored. For each center, at least one start visits and one closing visit must be carried out, which does not exclude the carrying out of one or more follow-up visits between these two mandatory visits.

The initial visit must be carried out before the inclusion of the first participant in that center; In it, the monitor will verify that the material to be used during the study has been received and that the personnel who will participate in the study activities have been trained on the study, as well as verify that the regulatory requirements and applicable standard operating procedures are met.

At the follow-up visit(s), the monitor will conduct a review of the study documents to confirm that the applicable research protocol and standard operating procedures are being followed, data completion is complete and timely, and that adverse event reports are being conducted appropriately. At each visit, the monitor will discuss the findings with the researcher and define the actions to be taken.

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

Details on monitoring are set out in the corresponding plan.

9.2 Audit and quality control

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

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

9.2.1 Pre-study audit

The research center included in the study will be subject to a feasibility visit prior to the selection of the center, where it will be verified that they meet 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 any audit or inspection is conducted, the investigator and the institution shall agree to allow the auditor/inspector direct access to all relevant documents and shall allocate their time and that of their staff to the auditor/inspector to discuss the findings and any pertinent problems. If the audit has not been scheduled by the sponsor, the facility must notify Laboratorios Sophia, S.A. de C.V. immediately.

10. Sample size calculation and statistical analysis

10.1 Sample Size Calculation

The sample size calculation was performed based on the primary objective of the study, to evaluate the safety and tolerability of the PRO-201 ophthalmic solution manufactured by Laboratorios Sophia, S.A. de C.V. on the ocular surface of ophthalmologically and clinically healthy subjects. To meet this objective, it is estimated that 29 subjects will contribute one eye (right eye [OD]) to the study.

10.1.1 Calculation methodology

Atropine sulfate 0.01% has sufficient scientific evidence that confirms its efficacy and safety as an adjuvant in the treatment to delay the progression of myopia in children. In the meta-analysis carried out by Zhao et al (2019), in a sample of 1079 subjects from 7 clinical studies (505 in the 0.01% atropine group and 574 in the control), the incidence of AEs in 0.01% atropine sulfate was statistically lower compared to the control group [OR=0.26, 95% CI (0.11, 0.61)] . While, in the review by Li et al (2019), children who received 0.01% atropine had a better balance between the use of the treatment-incidence of AD, being well tolerated at this concentration (only 2% had photophobia). On the other hand, in the meta-analysis by Wang et al (2020), no study reported severe/serious AEs after administration of 0.01% atropine. Finally, the meta-analysis by Huang et al. (2016), in agreement with these results, mentions that low-dose atropine (0.01%) is one of the most effective interventions with a minimal prevalence of clinical symptoms. Considering this information and the primary objective of the study, the following proposal was made for the sample size. [52, 53, 54, 55, 25] [52] [25][53][54]

For one-arm clinical studies (*single-arm trials*) where the primary endpoint depends on the duration of the study (e.g., survival rate, AD incidence, etc.), there are options available in the literature for sample size calculation. The most common options are the *log-rank* and assuming exponential distribution. Another option for calculation is to use a parametric exact test, following a Chi-square distribution. [56]

The probability that a subject will experience no improvement during the study is given by:

$$d = \int_0^\infty G(t) \cdot f_1(t)\, dt$$

Where *F1*(*t*) is *f*(*t*) with θ= θ1 (θ is a scale parameter, and β is the form of the parameter that determines the shape of the risk function [if β>1 the risk increases over time, and if β<1 the risk decreases over time, when β=1 represents a special case of exponential distribution with constant risk]). Dividing the number of events by *d* given the sample size adjusted for possible losses. [56]

Considering the OR calculated in the meta-analysis by Zhao et al, and the time of exposure to the product (14 days = 0.47 months) and follow-up time (21 days = 0.7 months), we can expect according to the simulation performed by Phadnis, a rate of 0.09 AE/subject in the study. Considering this incidence rate, in a sample of 24 subjects, 9% could present AE during one month of follow-up, see [52] [56]annex 16.5 Number of events/sample size for the exact method vs. the Wu method, for different values of the parameter β, cumulative time a, and tracking time f

10.1.2 Size Calculation

The PRO-201 ophthalmic solution is expected to be safe in its ophthalmic application when it has an incidence of unexpected, related AEs of less than 10%, and is tolerable when it has an incidence of photophobia that is less than 15% of the study population.

On this basis, a sample of 24 ophthalmologically and clinically healthy subjects was estimated. This value was increased by 20% considering possible losses. Based on this consideration, 29 subjects (who will contribute one eye, OD) are required to respond to the primary objective of the study.

10.2 Clinical Data Management

Clinical Data Management (CDM) *Clinical data management*), enables the generation of high-quality, reliable and statistically valuable data. CDM is the process of collecting, cleaning, and managing the information of the subjects of a study in accordance with regulatory standards (guidelines 21 CFR Part 11, ICH and GCP). It covers eCFR design, comments on the eCFR, database design, capture (*Data entry*), validation (*source document verification,* SDV), discrepancy management (*You want*), medical coding (*Medical Coding*), extraction (*Soft lock*) and database shutdown (*Hard Lock*). [57]

In accordance with roles and responsibilities, multiple users can be created, whose types of access to the eCRF can be limited to capture (principal investigator, IP), medical coding, database design, or quality control (*Quality Check*). The handling of discrepancies will be carried out based on the flow in Figure 5.[57, 58]

The following roles will be included in the CDM team:

- Data Manager,
- Database designer/programmer,
- Medical *coder*,
- Clinical Data Coordinator,
- Quality control, and
- Capturist (*data entry associate*). **[57]**

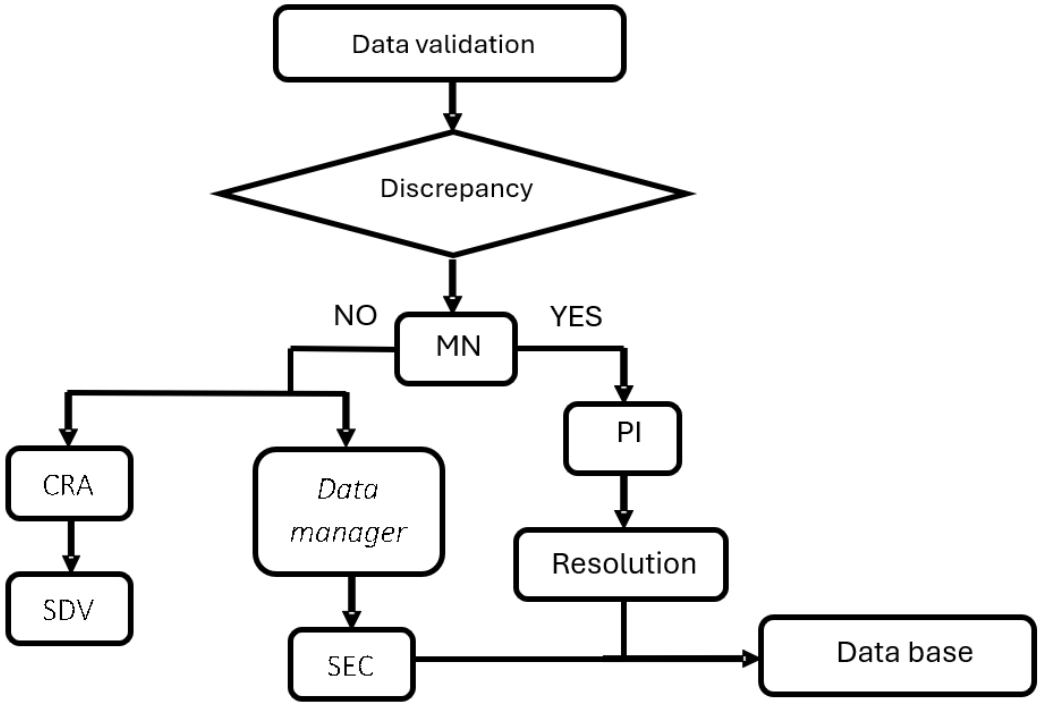


Figure 4. Management of discrepancies (MN, medical note; CRA, clinical monitor; SEC *self-evident correction*)

10.3 Statistical methodology

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

The statistical analysis will be carried out by personnel of Laboratorios Sophia, S.A. de C.V. The SPSS statistical package version 19.0 for Windows (IBM Corporation, Armonk, NY, USA) will be used. The coding will be done using consecutive numbers. The data will be collected and sorted in an Excel spreadsheet (Microsoft® Office). The data will then be exported to the SPSS package platform. The variables will be categorized according to their nature (see Board 4 Triangulation of concepts

The statistical tests used will be following the corresponding assumptions for non-parametric statistics.

10.3.1 Population Analysis

The statistical analysis will be presented to give a general summary of the subjects entered the study and a view of the safety and tolerability of its results. The data provided by the research site will be summarized for this purpose, according to its nature. The Shapiro-Wilk test will be performed to find out if the quantitative data are distributed in a normal way ($p>0.05$).

The results of the quantitative variables will be presented in measures of central tendency: mean, standard deviation, maximum and minimum. Changes in pupillary diameter, near visual acuity, BCVA and OCI will be expressed as continuous variables. Changes in IOP, HR, systemic blood pressure, and TF will be expressed as discrete variables.

The result of the nominal and ordinal qualitative variables will be presented in frequencies, proportions and/or percentages. For these, 2 x 2 frequency tables will be built. All percentages will be presented with decimal.

The level of difference to consider significance will be an alpha (α) of 0.05 or less. The triangulation between the variable type and the measurements is shown in the Board 4 Triangulation of concepts.

Board 4 Triangulation of concepts

Variable Type	Variable	A1	A2	B1	C1	C2	D1	D2	D3	E1	E2	E3	E4	E5
Selection														
A1	Demographic	D												
A2	Medical History/Selection Criteria		D											
Basal														
B1	Comprehensive ophthalmological evaluation		DT	D	TB			TB						
Safety & Tolerability														
C1	Incidence of AE*				TB			TB						
C2	Incidence of photophobia				TB	TB		TB				T		
Secondary Security Outcome														
D1	Pupillary diameter						B	TB						
D2	Incidence of AD				TB	TB		TB	TB	TB	TB	TB	TB	
D3	Near visual acuity		D					TB	B					
Exploratory														
E1	BCAV		D					TB		B				
E2	PIO		D					TB			B			
E3	Corneal and conjunctival stains with fluorescein and lysmine green		D					TB				B		
E4	Vitals signs		D					TB					B	
E5	OCI		D											B

Abbreviations: BCVA, best corrected visual acuity; B, bivariate analysis; D, descriptive statistics; AE, adverse event; OCI, ocular comfort index; M, multivariate analysis; IOP, intraocular pressure; T, 2x2 contingency table. *Unexpected-related AEs.

10.3.2 Safety and tolerability analysis

10.3.2.1 Analysis for Primary Variables

An analysis of safety and tolerability outcomes will be carried out in the safety population, defined as any subject who has received at least one dose of the investigational product (PRO-201) regardless of adherence to it or adherence to the protocol (intention-to-treat population, ITT).

The statistical analysis for the primary variables of a quantitative type will be estimated by means of the Kolmogorov-Smirnov statistic for the difference in measurements, once the values have been adjusted with respect to their baseline within everyone. In cases where applicable, the nonparametric Mann-Whitney U test will be used.

For conjunctival hyperemia, Pearson's X2 or Fisher's exact test will be used at 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 carried out as long as they have the necessary measurements to do so.

10.3.3 Exploratory Analysis

The analysis of exploratory variables will be carried out in those subjects who conclude their participation without deviations from the study protocol (population by protocol, PP). The same primary analysis will be carried out if they have the necessary measurements to do so.

10.4 Procedure for handling missing data

There is no imputation procedure for missing data.

11. Ethical considerations

11.1 Approval of the committees

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

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

The study will not begin at the research center if there are no confidentiality agreements and economic proposals from each of the principal investigators, duly signed and without having previously obtained the favorable opinion and/or approval of the Research Ethics Committees, Research Committees, and when applicable by the Biosafety Committee. Corresponding.

The study will not begin without having complied with the relevant local, national or international regulatory requirements and without having the corresponding health authorization.

The study is a study with a 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 of January 6, 1987.

11.2 Amendments to the protocol

The amendment procedure will be pertinent when there is a need to make any change to a document that is part of the research project or protocol, derived from variations in the methodological structure, substitution of the principal investigator or in the face of the identification of risks in the research subjects. The documents subject to amendment will be protocol, letter of informed

consent, researcher's manual, documents for the subject, measurement scales and schedule of activities.

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 COFEPRIS.

Amendments that substantially modify the protocol confer an additional or different risk on the research subjects must be approved by the Committees. It is the investigator's responsibility to act in situations that require immediate action to avoid unnecessary harm to study participants.

The principal investigator is responsible for communicating to the Research Ethics Committee any amendments to the protocol that may eventually affect the rights, safety, or welfare of research participants. Likewise, it must inform us of any situation or new knowledge that will show a greater risk for the participants, the premature termination or suspension of the study, the reasons and the results obtained so far. It must also report on the conclusion of the study upon completion of the research protocol.

11.3 Early Study Termination

The study may be suspended or terminated prematurely if there is a sufficiently reasonable cause. Written notice, documenting the reason for the suspension or early termination, shall be delivered by the party enforcing the suspension. The PI should promptly inform the study participants, the IC, and the IRC providing the reasons.

Situations in which study suspension or early termination will be contemplated 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 (COFEPRIS) considered it for security alerts.
3. The Sponsor determined it for its convenience or eventualities such as financial support, manufacturing errors, etc.
4. The identification of unexpected risks to the participants, which are significant or unacceptable.
5. Obtaining new relevant safety information.
6. Insufficient adherence to the requirements of the protocol.
7. The data obtained is not assessable or is 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 corrected; if this justification is sufficient for the sponsor, CI, CEI and regulatory authorities.

11.4 Informed Consent

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

The FCI will be considered as 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 are submitted to the appropriate approvals (the same as those to which the original informed consent letter was submitted) and that the most current approved version is the one 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, a letter of informed consent must be signed.

Written consent documents will incorporate the elements of informed consent described in the Declaration of Helsinki and the ICH Guide to Good Clinical Practice and will follow all applicable laws and regulations.

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

This information will be in a language understandable to the subject, it will be explained to the subject that he or she has the right to interrupt his or her participation in the study at any stage, without this affecting the relationship with the researcher and/or his or her future assistance. Informed consent will be put to the consideration of the potential participant; He must have enough time to analyze each one of the aspects mentioned above and in case he has any doubts, it will be clarified by the person in charge of obtaining the informed consent.

Once the participant agrees to participate in the study, he/she must sign and date the letter of informed consent in the presence of two witnesses who are or are not related to the study subject, who will participate during the informed consent process and will sign guarantee that the process was carried out prior to any study procedure. that the information of the study was clearly explained, and doubts were clarified if any.

If a subject is illiterate, acceptance will be with his or her fingerprint, and if the subject is unable to give adequate written informed consent, a representative of the "legally authorized" subject may provide such consent for the subject in compliance with applicable laws and regulations.

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

The FCI must be signed in duplicate by all those involved, one copy will be filed in the researcher's folder, and the other will be given to the participant. The PI or delegated personnel must document the process of obtaining the Informed Consent by means of a detailed, precise and contemporary medical note, specifying the signed version, the date on which 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 an additional risk that should be considered apart from the procedures listed for 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 Research Ethics Committees and COFEPRIS.

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

A process of re-consent for each subject affected by the amendment must be carried out under the same conditions as those described above, to communicate the new information contained in the document to them in a timely manner. The subject will be given a signed original of the amendment, and the researcher will keep the second original.

11.5 Confidentiality

All documents and information provided to the research center by the sponsor are strictly confidential. PI expressly agrees that data about your professional and clinical experience, provided to the sponsor on paper and stored in electronic form, is solely for use related to your activities with the clinical trial sponsor, in accordance with Good Clinical Practice.

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

The clinical study protocol provided to the PI may be used by the PI and his team to obtain the informed consent of the subjects for the study. The clinical trial protocol, as well as any information taken from it, should not be disclosed to other parties without the written permission of the sponsor.

The PI will not disclose any information without the prior written consent of Laboratorios Sophia, S.A. de C.V., except to the representatives of the Competent Authorities, and only at their request. In the latter case, the investigator undertakes to inform Laboratorios Sophia, S.A. de C.V. before disclosing the information to these authorities.

The PI will fill out and maintain a record of the selection of the subjects, as well as the identification and enrollment list of each of the subjects participating in the study. The investigator agrees to give on-site access to the auditor and/or representatives of the Competent Authorities. The information will be processed in compliance with professional secrecy.

In the eCRF and all communications related to study subjects, they will identify them only by the study subject's identification number, either by the scrutiny number or the assignment number. The information collected in this study will be exchanged between the sponsor and the research center and must be treated confidentially. The Health Authority, the CEI, 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, in no case will they contain information on the identification of the study subjects. If the results of the study are published, no personal information on the study subjects will be revealed.

The protection of personal data will be carried out in accordance with the corresponding regulations in force.

11.6 Conflict of interest

The independence of the conduct of the study and its results from any current or perceived external influences is critical. For this reason, any current conflict of interest of any person who has a role in design, conduct, analysis, publication or any aspect of this study will be declared. Furthermore, those who have a perceived conflict of interest will be asked to handle it in a manner appropriate to their participation in the study.

11.6.1 Declaration of Interests

The PI undertakes to make a declaration of financial interests as well as conflict of interest prior to the start of the study.

11.7 Access to Information

The final database of the study will be the property of Laboratorios Sophia, S.A. de C.V. and its access will be restricted. The PI will not have access to it, unless it has prior written authorization from the sponsor.

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

11.8 Ancillary and post-study care

Upon completion of the study and closure of adverse events in accordance with section: 8, the sponsor shall 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 the PRO-201 ophthalmic solution, prepared by Laboratorios Sophia, S.A. de C.V. on the ocular surface of ophthalmologically and clinically healthy subjects", and number: SOPH201-0521/I has no biosafety implications, since infectious and contagious biological material will NOT be used; pathogenic strains of bacteria or parasites; viruses of any kind; radioactive material of any kind; genetically modified animals and/or cells and/or plants; toxic, dangerous or explosive substances; any other material that endangers the health or physical integrity of the research center's staff or research subjects or affects the environment. It is also declared that this project will not carry out cell, tissue or organ transplant procedures, or cell therapy, nor will laboratories, farm or wildlife animals be used.

13. Posting Policy

13.1 Final Report

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

13.2 Communication of results

Regardless of the results of the study, Laboratorios Sophia, S.A. de C.V., is committed to communicating the final report of the study to the principal investigators and COFEPRIS. These results will also be shared with the research committee and the IRC. It will be the responsibility of the PI to communicate it to the research subjects.

Laboratorios Sophia, S.A. de C.V. will always maintain the rights over the publication and disclosure of the information contained.

13.3 Publication of results

Laboratorios Sophia, S.A. de C.V., acting as the sponsor of the study, assumes full responsibility for its function and retains exclusive ownership rights over the results of the study, which it may use in any way it deems appropriate.

The PI undertakes not to publish or communicate data collected from the study, unless there is the prior written agreement of Laboratorios Sophia, S.A. de C.V. Any manuscript derived from the data obtained with this protocol must be submitted to review by the sponsor before any attempt to submit it for publication in any scientific journal or congress.

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

The assignment of authorship of publications, which is the responsibility of the sponsor, will be the prerogative of the latter. However, the express authorization of the people who are invited to participate as authors must be obtained. Authors have the right to review the manuscript prior to its publication, as well as to issue comments and suggestions in this regard, such comments must be delivered within the first 15 calendar days from the date on which the project is received.

14. Financing and Insurance

14.1 Compensation to Study Participants

Subjects who participate in the study will receive financial compensation for their participation in the study.

14.2 Insurance for Study Participants

The subjects participating in the study will sign the informed consent form, which specifies that Laboratorios Sophia, S.A. de C.V. agrees to pay for immediate treatment resulting from injuries or illnesses caused by the investigational products until their resolution, according to medical criteria.

All study participants will be entitled to the coverage of a liability policy, contracted by Laboratorios Sophia, S.A de C.V. The information of the policy contracted will be available at the research center. In the event of a medical emergency, the research center must have personnel, material, equipment and procedures for its immediate management.

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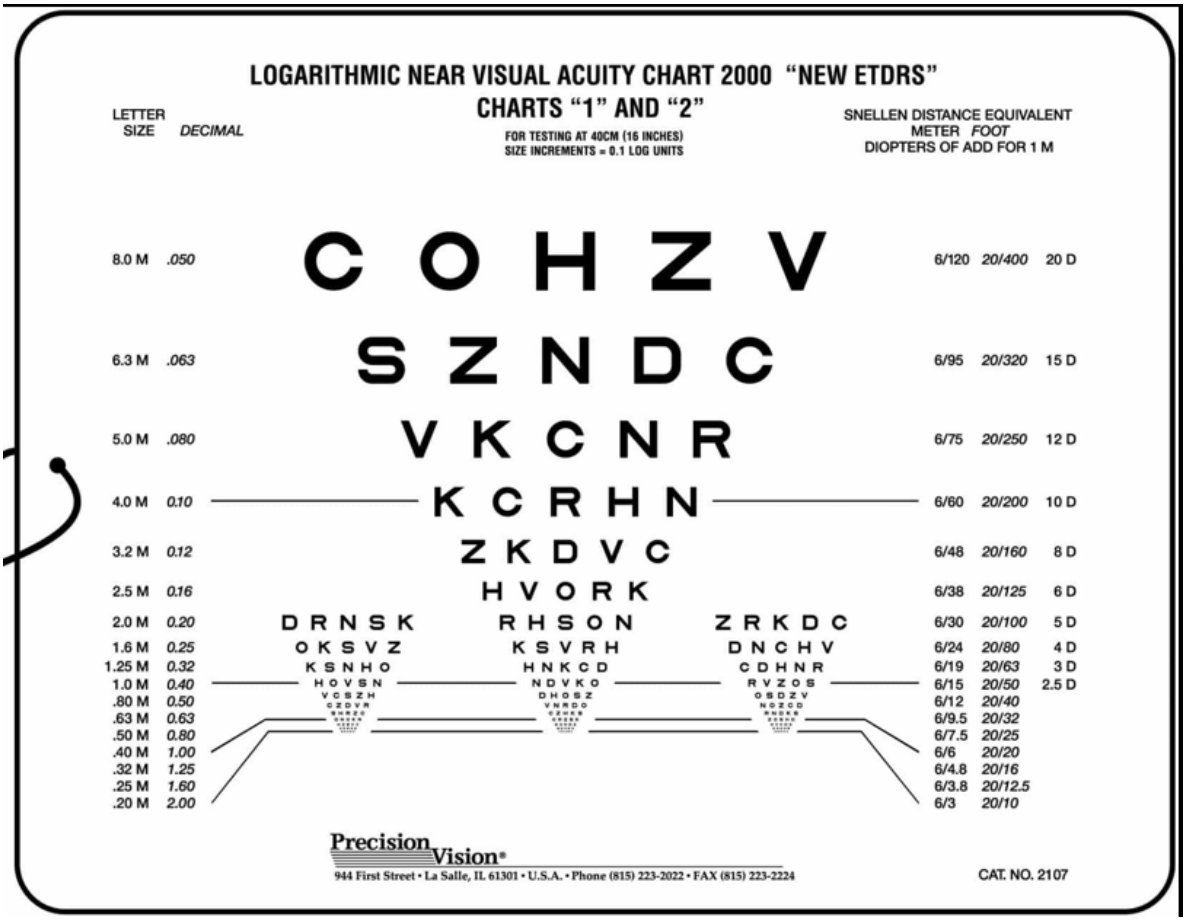
16. Annexes

16.1 ETDRS Primer for Near Vision



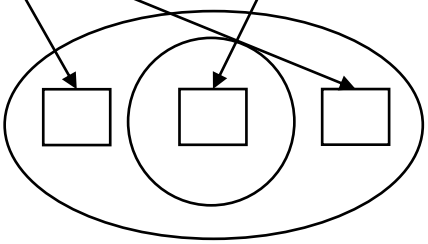
The above annex is a representation of the ETDRS primer for near vision, it is possible that the booklet designated for the study has variations in format and translation.

16.2 SICCA Eye Staining Rating (modified from Whitcher et al, 2010)

Score by staining pattern:

Grade Points	Green Lysmine (conjunctiva only)
0	0-9
1	10-32
2	33-100
3	>100

Grade Points	Fluorescein (cornea only)
0	0
1	1-5
2	6-30
3	>30



Additional points for fluorescein staining only:

+1 if there are confluent staining patches

+1 if there is staining in the pupillary area

16.3 Ocular Comfort Index (OCI)

Study No SOPH201-0521/I **Date**_____
Subject initials_____ **Participant number** 201- -

Instructions:

This questionnaire was designed to grade the comfort of your eyes.

For each question, please circle your answer.

Example: In the last week, how often were your eyes red?

(Never 0 1 2 3 4 5 6 (Always)

There are no right or wrong answers. Do not spend too long on any one question.

1. In the last week, how often did your eyes feel dry?

   (Never 0 1 2 3 4 5 6 (Always)

   When your eyes felt dry, typically, how intense was the dryness?

   (Never 0 1 2 3 4 5 6 (Always)

2. In the last week, how often did your eyes feel gritty?

   (Never 0 1 2 3 4 5 6 (Always)

   When your eyes felt gritty, typically, how intense was the grittiness?

   (Never 0 1 2 3 4 5 6 (Always)

3. In the last week, how often did your eyes feel gritty?

   (Never 0 1 2 3 4 5 6 (Always)

   When your eyes stung, typically, how intense was the stinging?

   (Never 0 1 2 3 4 5 6 (Always)

4. In the last week, how often did your eyes feel tired?

   (Never 0 1 2 3 4 5 6 (Always)

   When your eyes felt tired, typically, how intense was the tiredness?

   (Never 0 1 2 3 4 5 6 (Always)

5. In the last week, how often did your eyes feel painful?

   (Never 0 1 2 3 4 5 6 (Always)

   When your eyes felt painful, typically, how intense was the pain?

   (Never 0 1 2 3 4 5 6 (Always)

6. In the last week, how often did your eyes itch?

   (Never 0 1 2 3 4 5 6 (Always)

   When your eyes itched, typically, how intense was the itching?

   (Never 0 1 2 3 4 5 6 (Always)

16.4 Fraction to LogMAR conversion table.

LogMAR	Snellen (feet)	Snellen (meters)	Decimal
1.0	20/200	6/60	0.10
0.9	20/150	-	-
0.8	20/120	6/36	0.15
0.7	20/100	-	0.20
0.6	20/80	6/24	-
0.5	20/60	6/18	0.30
0.4	20/50	-	0.40
0.3	20/40	6/12	0.50
0.2	20/30	6/9	-
0.1	20/25	-	0.75
0.0	20/20	6/6	1.00
-0.1	20/15	6/5	-
-0.2	-	6/4	1.50

16.5 Number of events/sample size for the exact method vs. the Wu method, for different values of the parameter β, cumulative time a, and tracking time f

Study specific parameters: median time under H_0 = 2.5 months; median time under H_A = 3.75 months; alpha = 0.05 (one-sided); target power = 0.80;

Shape parameter	a	Method	f=1	f = 2	f = 4	f = 6	f = 9	f = 12
β = 1.50	a = 0	Exact	16/176	16/68	16/30	16/22	16/18	16/17
		Wu	14/151	14/61	16/30	17/23	19/21	21/21
	a = 3	Exact	16/52	16/35	16/23	16/19	16/17	16/17
		Wu	15/48	16/34	17/25	19/22	20/21	21/21
	a = 6	Exact	16/32	16/26	16/21	16/18	16/17	16/17
		Wu	17/33	17/28	18/23	19/21	20/21	21/21
	a = 9	Exact	16/26	16/23	16/19	16/18	16/17	16/17
		Wu	18/28	18/25	19/22	20/21	21/21	21/21
	a = 12	Exact	16/23	16/21	16/18	16/17	16/17	16/17
		Wu	19/26	19/24	20/22	20/21	21/21	21/21
	a = 15	Exact	16/21	16/20	16/18	16/17	16/17	16/16
		Wu	19/25	19/23	20/22	20/21	21/21	21/21

a= Cumulative time in months, f= follow-up time in months.
The term "exact" refers to the exact calculation using a Chi-square distribution.
*Table taken and partially adapted from Phadnis MA, 2019.
Annex taken from Phanis, 2019 [56]