Creation date: july de 2016

Phase I clinical study, to evaluate the safety and tolerability of the preservative-free ophthalmic solution PRO-087 versus Xyel Ofteno® and Systane Ultra®, on the ocular surface of ophthalmologically and clinically healthy subjects

Protocol code:SOPH087-0616/I

Protocol version: 1.0

Date of the version: 29/06/2016

Registry: 173301410A0014/2017

Sponsor: Sophia Laboratories, S.A. of C.V.

1. Summary

<table>
<tr><td colspan="3">Title of the study:
Phase I clinical study, to evaluate the safety and tolerability of the preservative-free ophthalmic solution PRO-087 versus Xyel Ofteno® and Systane Ultra®, on the ocular surface of ophthalmological and clinically healthy subjects.</td></tr>
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1.0</td><td>Date of the version:
29/06/2016</td></tr>
<tr><td colspan="3">Therapeutic Indication:
Eye lubricant</td></tr>
<tr><td>Study period:
3 to 4 months</td><td colspan="2">Development phase: I</td></tr>
<tr><td colspan="3">Goals:
To evaluate the safety and tolerability of the preservative-free formulation PRO-087 manufactured by Sophia Laboratories, S.A. of C.V. on the ocular surface of clinically healthy subjects.</td></tr>
<tr><td colspan="3">Hypothesis:
To evaluate the safety and tolerability of the preservative-free formulation PRO-087 manufactured by Sophia Laboratories, S.A. of C.V. on the ocular surface of clinically healthy subjects.</td></tr>
<tr><td colspan="3">Methodology:
Phase I clinical trial, controlled, of parallel groups, double blind, randomized, exploratory.</td></tr>
<tr><td colspan="3">Number of patients:
30 subjects, divided into 3 groups [10 subjects (20 eyes) exposed by group]</td></tr>
<tr><td colspan="3">Diagnosis and main inclusion criteria:
Systemically and ophthalmologically healthy subjects
- Signed informed consent.
- Age between 18 to 40 years.
-Both genders
- Blood tests [complete blood count (BHC), three element blood chemistry (QS) and liver function tests (PFH)] within normal parameters
- Visual capacity 20/30 or better</td></tr>
</table>

Test product, dose and route of administration, lot number:

- PRO-087. Chondroitin sulfate 0.18% / 0.1% sodium hyaluronate, ophthalmic solution free of preservatives. Prepared by Sophia Laboratories, S.A. of C.V., Zapopan, Jalisco, Mexico.
    - Dosage: 1 drop 4 times a day during the waking period, both eyes
    - Route of administration: topical ophthalmic

Duration of treatment: 10 days

Reference product, dose and route of administration, lot:

1. **Xyel Ofteno®.** Xantana gum 0.09% / Chondroitin sulfate 0.1% / preservative-free ophthalmic solution. Prepared by Sophia Laboratories, S.A. of C.V., Zapopan, Jalisco, Mexico.
    a. Dosage: 1 drop 4 times a day during the waking period, both eyes
    b. Route of administration: topical ophthalmic
2. **Systane Ultra®.** Polyethylene glycol 400 0.4%, propylene glycol 0.3%. Prepared by Alcon Laboratories, Inc.
    a. Dosage: 1 drop 4 times a day during the waking period, both eyes
    b. Route of administration: topical ophthalmic

Evaluation criteria:

Primary security outcome variables:

- Density of goblet cells.
- Presence of adverse events.
- Intraocular pressure.
- Visual ability
- Laboratory tests: BHc, QS and PFH.
- Epithelial defects in cornea and conjunctiva.
- Ophthalmological signs: conjunctival hyperemia, chemosis.

Secondary outcome variables:

- Rupture time of the tear film
- Life signs: FC, FR, TAS.
- Subsequent segment

Primary outcome variables of tolerability:

- Burning
- Foreign body sensation
- Itching
- Eye comfort index

Statistical methodology:

The data will be expressed with measures of central tendency: mean and standard deviation for the quantitative variables. The qualitative variables will be presented in frequencies and percentages. The statistical analysis will be carried out through the Kruskal-Wallis test for quantitative variables. The difference between the qualitative variables will be analyzed by means of X2 (Chi2). An alpha ≤ 0.05 will be considered significant.

2. Content

Index of tables and figures

3. Index of abbreviations

ALT	Alanino transferase
AST	Aspartate transferase
BAK	Benzalkonium chloride
BD	Bilirubin direct
BI	Bilirubin indirect
BHc	Complete blood count
BPC	Good clinical practices
BT	Total Bilirubin
CV	Visual capacity
CCI	informed consent letter
CIC	Cytology by conjunctival impression
IEC	Research Ethics Committee
CI	Informed Consent
CRF	Case Report Form (Case Report Form)
EA / EAS	Adverse event / serious adverse event
FDA	Food and Drug Administration (Food and Drug Administration)
FC	Heart rate
FR	Respiratory frequency
GAG	Glycosaminoglycans
ICH	International Conference on Harmonization
ICO	Eye comfort index
IP	Principal investigator of the clinical study
PFH	Liver function tests
IOP	intraocular pressure
TAS	Systemic blood pressure

TF	Fluorescein staining
TVL	Green lysine stain
QS	Blood chemistry
TVL	Green lysine stain
QS	Blood chemistry

4. Administrative structure of the study

The administrative structure of the sponsoring party, corresponding to Sophia Laboratories, S.A. of C.V. is shown in **Table 1. Administrative structure**

Function	Contact/name	Affiliation ¥
Medical responsible for the study	Dr. Leopoldo Martín Baiza Durán leopoldo.baiza@sophia.com.mx	Medical Director and Regulatory Affairs
Director of the study	Dr. Aldo Arturo Oregón Miranda aldo.oregon@sophia.com.mx	Clinical Operations Manager
Scientific Comittee	Dr. Oscar Olvera Montaño oscar.olvera@sophia.com.mx	Ophthalmologist Investigator
Scientific Comittee	Dr. en C. Arieh Roldán Mercado Sesma arieh.mercado@sophia.com.mx	Medical Editor
Coordinator of regulatory procedures	LN. Ana Isabel Alcaraz Ledón ana.alcaraz@sophia.com.mx	Specialist in the beginning of clinical studies
Monitoring coordinator	QFB Virginia Manuela Villa Félix virgina.villa@sophia.com.mx	Monitor coordinator
International monitor	M. en F. Sandra Carolina Gómez Méndez sandra.gomez@sophia.com.mx	Senior Monitor
Monitor	QFB Jessica Lizette Mejía Gutiérrez jessica.mejia@sophia.com.mx	Senior Monitor
Monitor	LP María Angela González Ávila maria.gonzalez@sophia.com.mx	Junior Monitor
Monitor	QFB Lizeth de Jesús Pérez Lerma lizeth.perez@sophia.com.mx	Junior Monitor
Monitor	LN Pilar Carolina Castro Mata pilar.castro@sophia.com.mx	Junior Monitor
Administrative Assistant	LAET Maira Alejandra Ramírez Velazco alejandra.ramirez@sophia.com.mx	Clinical Operations Assistant

¥ Employees of Sophia Laboratories, S.A. of C.V Av. Paseo del Norte No.5255, Col. Guadalajara Technology Park, Carretera Guadalajara-Nogales Km13.5 C.P45010 Zapopan, Jalisco, Mexico Tel +52 (33) 3000 4200

Table 1. Administrative structure

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5. Introduction

5.1 Theoretical framework

Dry eye disease (DED) is a frequent ocular condition that significantly decreases quality of life and affects 6-34% of the world's adult population. [1] [2] Although there is no formal study on the prevalence of the disease in Latin American countries, several reports agree that there is a higher prevalence of severe symptoms and clinical diagnosis of DED in the Hispanic population when compared with the Caucasian population. [2] [3]

DED is an alteration of the tear film that results in damage to the ocular surface and is associated with symptoms of ocular discomfort. The DED has also been called keratoconjunctivitis sicca (KCS), sicca syndrome, xerophtalmia, dry eye syndrome, ocular surface disease, tear film dysfunction syndrome (SDPL) or simply dry eye. [4] [5] Strictly some of these terms are not synonymous, since for example, the DED can be presented without keratitis, KCS; This protocol will be used as a synonym of the DED to the SDPL, assuming they are interchangeable concepts and adopting the definition of the International Dry Eye Seminar 2007 (DEWS, for its acronym in English of International Dry Eye Workshop):

> *Multifactorial disease of the tear and the ocular surface that results in symptoms of discomfort, visual alterations and instability of the tear film with potential damage to the ocular surface. It is accompanied by an increase in the osmolarity of the tear film and inflammation of the ocular surface.* [4]

The SDPL is an alteration of the functional lacrimal unit (ULF), an integral system comprised of lacrimal glands, ocular surface (cornea, conjunctiva and Meibomian glands), eyelids and the sensory motor nerves that interconnect them. [6]

This ULF controls the main components of the tear film and regulates them in response to environmental, endocrinological and cortical influences. Its main function is to preserve the integrity of the tear film, the transparency of the cornea and the quality of the image projected onto the retina. [6] [7] [8] [9]

The damage or alteration to any component of the ULF can destabilize the tear film and lead to an eye surface disease that is expressed as SDPL. The stability of the tear film, distinctive of a normal eye, is threatened when the interactions between the stabilizing constituents of the tear film are compromised by decreased secretion, delayed clearance and an altered tear composition. Inflammation is a secondary consequence. The reflex tear secretion, in response to irritation, is considered to be the initial compensatory mechanism, but, over time, the inflammation that accompanies secretory dysfunction and decreased corneal sensitivity compromises the reflex response and results in instability of the even bigger tear film. It is considered that the disturbance of the UFL plays a very important role in the evolution of different forms of dry eye. [4]

The tear film is a highly specialized and carefully structured moisturizing layer that covers the cornea and the conjunctiva. It is classically described as a trilaminar structure composed of a lipid surface layer, an aqueous intermediate layer and a mucinous inner layer. [10] The tear film has four main functions: 1) Maintain a regular optical surface, 2) Do not allow friction between structures of the

ocular surface, 3) Nourish the cornea and 4) First line of defense against ocular surface infections.[11]

Based on the models of the "microstructure" of the tear film and its interface with the cells of the ocular surface, the mucin layer should always be present in a healthy tear film. [10] This layer provides support to the rest of the tear film on the ocular surface, helping to keep it moisturized and lubricated. [12] The epithelial cell layer of the conjunctiva includes the goblet cells, which are mucin excretors. The main source of mucin for the tear film are these cells. [11]

Within the framework of the DEWS, the etiopathogenic classification of the SDPL was established, which is summarized. This classification aims to provide a more up-to-date understanding of the SDPL. It is divided into two main classes: aqueous and evaporative deficiency. The category of aqueous deficiency refers mainly to a lack of tear secretion. The evaporative class has been subdivided to distinguish from causes that depend on intrinsic conditions of the eyelids and ocular surface and those that arise from extrinsic influences.

The SDPL can start in either of the two classes, but these are not mutually exclusive. It is recognized that a disease can start in a main class and coexist or even lead to events that produce SDPL by a mechanism of another kind. This is part of a vicious cycle of interactions that can amplify the severity. An example of this may be that all forms of SDPL cause loss of goblet cells, which over time will contribute to a loss of tear film stability, surface damage and evaporative water loss. [4]

Many pathophysiological mechanisms of the SDPL stimulate the sensory nerves of the cornea, so the SDPL is described as a "symptomatic disease". [13] [14] [15] In most patients there is a relationship between symptoms and clinical signs, nevertheless it is also recognized that in some patients the severity of the symptoms does not correspond to the clinical signs of the disease. [16] [14] [17]

The classification of the SDPL according to its severity currently represents a challenge, since there is no gold standard to determine it; notwithstanding, the severity of the disease is one of the most relevant factors when considering the therapeutic options for the SDPL. [18] In 2006 a panel of specialists, called the Delphi Panel, issued a classification of severity later adopted by the DEWS. The severity was classified into four levels, based on the increase in frequency and intensity of various signs and symptoms. [18] See **¡Error! No se encuentra el origen de la referencia.**

There are other systems to classify the SDPL severity referred by other authors,[19] [20] [21] [22] among them the algorithm of the European consensus of the ODISSEY group which was designed to classify as severe patients with discordant SDPL and therefore, more complex to classify. [23]

In addition to the evaluation parameters of the SDPL, among which a greater correlation has been found, OSDI, TRL, and Schirmer's test, cytology by conjunctive impression (CIC) has been used recently, which is a minimally invasive technique that allows the analysis of the disease in a clinical setting by evaluating mainly the characteristics of goblet cells. CIC can be used for diagnostic purposes, to understand the mechanism of the disease and to evaluate the effectiveness of a treatment. [24]

Figure 1. Classification of SDPL by etiology.

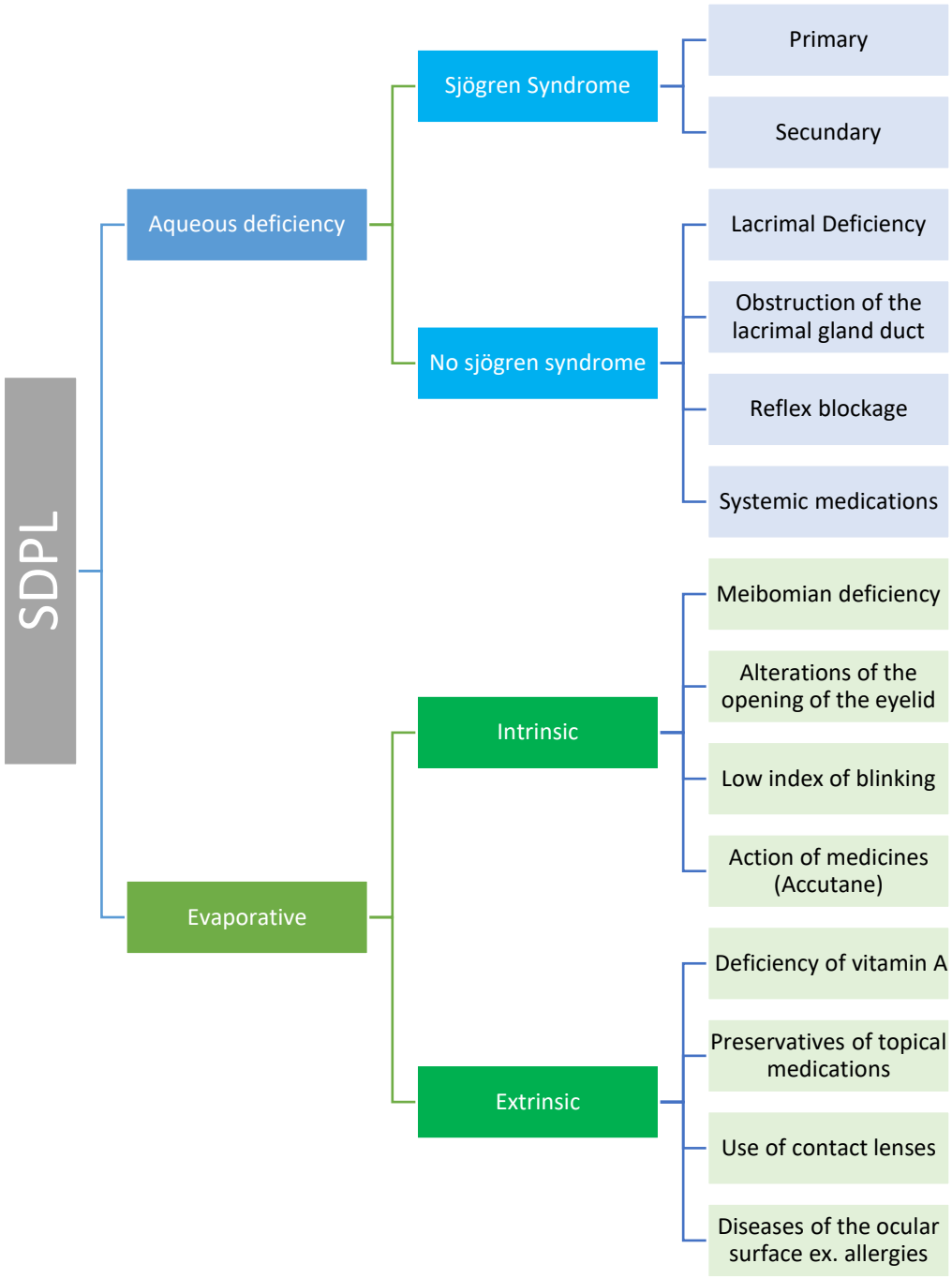


Table 2. Classification of the SDPL by severity

Grade	1 Mild	2 Moderate	3 Severate	4* Incapacitator
Discomfort, severity and frequency	Occurs mildly and / or episodically; under conditions of environmental stress	Occurs chronically or episodically, with or without environmental stress	Occurs frequently and / or constantly without stress	Is constant
Visual symptoms	None or episodic mild fatigue	Disturbances, activity limitation in an episodic manner	Disturbances, activity limitations in a chronic and / or constant way	Constant and / or disabling
Conjunctival injection	None to mild	None to mild	+	+/++
Conjunctival stain	None to mild	Variable	Moderate to marked	Marked
Corneal staining	None to mild	Variable	Central marking	Severe and disseminated dot erosion
Lacrimal / corneal signs	None	Mild debris, meniscus diminution	Filamentous keratitis, lacrimal debris and mucinous accumulations	Filamentous keratitis, lacrimal debris, mucinous accumulations, ulcer
Meibomian glands / eyelids	Meibomitis present in a variable way	Meibomitis present in a variable way	Frequent	Trichiasis, keratinization, simblefaron
TRL	Variable	≤ 10	≤ 5	Immediate
Schirmer I	Variable	≤ 10	≤ 5	≤ 2

* It must have signs and symptoms.
Adapted from Behrens A, Doyle JJ, Stern L, et al.[18]

The treatment currently available for the SDPL can be divided into:

a) Lacrimal supplements: lubricants.
b) Tears retention: lacrimal dot occlusion, contact lenses.
c) Secretion stimulation: secretagogues.
d) Biological substitutes: autologous serum, autologous salivary gland.
e) Anti-inflammatories: cyclosporine, steroids, tetracyclines.
f) Essential fatty acids
g) Environmental strategies

The treatment recommendations of the American Academy of Ophthalmology and the Guidelines for the Dry Eye of the International Working Group, favored by the DEWS, are based on severity See **¡Error! No se encuentra el origen de la referencia.**. The recommendations can be modified by the ophthalmologist, based on the clinical experience and the individual profile of your patient. [18] [25] [26]

Table 3. Treatment recommendations for the SDPL

Level 1:
Education and dietary and environmental modifications
Elimination of systemic medications that alter the UFL
Lubricants
Eyelid treatment
Level 2:
If the treatments of level 1 are insufficient, add:
Anti-inflammatories
Tetracyclines
Closing the puncta with silicone tips
Secretagogues
Level 3:
If the treatments of level 2 are insufficient, add:
Autologous serum
Contact lenses
Permanent closure of the lacrimal dots
Level 4:
If level 3 treatments are insufficient, add:
Systemic anti-inflammatories
Surgery (tarsorraphy, transplantation: mucous membrane, salivary gland, amniotic membrane)
Modified from the Guidelines for the Dry Eye of the International Working Group [18]

The main objective in the care of patients with SDPL is to improve the patient's ocular comfort and quality of life, in addition to returning the ocular surface and the tear film to its state of homeostasis. Although the symptoms are rarely eliminated, they can often be diminished, resulting in an improvement in the quality of life. [26]

Ocular lubricants are the first line of management for the SDPL and a constant in all levels of treatment. They are characterized by hypotonic or isotonic solutions, which contain electrolytes, surfactants and various types of viscous agents. The main variables in the formulations of ocular lubricants are in relation to the selection or concentration of electrolytes, the osmolarity, the type of visco-polymeric system, the presence or absence of preservatives. [26]

5.2 Definition of the problem and fundamental reason

There are few effective treatments for the treatment of SDPL. The clinical development of new treatments is slow because the pathogenesis of SDPL is multiple and its semiotics variable. The different phases of treatment have as a common denominator the use of ocular lubricants.

Although there is a great variety of topical lubricants, with different viscous agents, there is no evidence that one is better than another. The elimination of preservatives, mainly benzalkonium chloride (BAK, for its acronym in English benzalkonium chloride) or the development of new less toxic preservatives have made eye lubricants more tolerable.

Although ocular lubricants have not been shown to be sufficient to completely resolve the alteration of the ocular surface and the inflammation seen in patients with SDPL, they have been shown to provide protection to the ocular surface and to diminish the symptomatology and clinical findings.

The combination of sodium hyaluronate (HS) and chondroitin sulfate (CS), which are biopolymers, glycosaminoglycans (GAG), constituents of the extracellular matrix, contributes to PRO-087 the viscoelastic and water retention properties, to function as a lubricant effective that protects the ocular surface and reconstitutes the tear film.

5.3 Background

In Mexico and other South American countries, Humylub Ofteno® is available, which is the association of HS and CS itself that contains PRO-087, but with BAK as conservative. Humylub Ofteno® has been registered in Mexico since July 2007 and there have been no reports of adverse reactions associated with the use of Humylub Ofteno®, maintaining a good safety profile. [27]

5.3.1 Sodium hyaluronate

It is a biopolymer, disaccharide of the GAG family, formed by the alternating sequence of N-acetyl-D-glucosamine and glucuronate in linear chains. In physiological solvents it forms spirals; configuration determined by its viscosity. It is a constituent of the extracellular matrix, connective tissue, vitreous humor, umbilical cord, synovial fluid, skin, etc. It can be constituted by more than 10,000 pairs of disaccharides. At concentrations above 0.1% sodium hyaluronate constitutes a network. The diffusion rate through the network is inversely related to the size of the polysaccharide molecules, which are stable. Proteoglycans contribute to mechanical and elastic properties. Numerous authors have reported that the HS has a high capacity to retain water, it has been established that 1 gr of HS can retain up to 6L of water. [28]

Sodium hyaluronate is synthesized on the inner side of the plasma membrane as a linear polymer, in contrast to other GAGs which are synthesized by enzymes in the Golgi apparatus. The enzymes for the synthesis of sodium hyaluronate are hyaluronate and glucosyltranferases, which coordinately polymerize and translocate sodium hyaluronate out of the cell into the extracellular matrix. [29]

5.3.1.1 Eyeball pharmacokinetics

Route of administration: Ophthalmic.

Release: immediate.

Absorption: HS absorption through the cornea has not been reported when applied to the ocular surface. Pharmacokinetic studies performed in patients with dry eyes showed that the HS solution reached its maximum concentration in 10 minutes and is widely distributed on the ocular surface.

Metabolism: it is biotransformed by hyaluronidases.

Elimination: it is eliminated from this compartment through the lacrimal sac and the lacrimal duct without intraocular absorption, in approximately 45 minutes. [30] Based on the results of preclinical research in rabbits developed by Laboratorios Sophia SA de CV, the ocular half-life correlates with the volume of the formula.

5.3.2 Chondroitin sulfate

It is part of the group of GAGs, like sodium hyaluronate, is a mucopolysaccharide found in the extracellular matrix of connective tissues, including the vitreous, cornea and aqueous humor. GAGs are high molecular weight aggregates called proteoglycans. These proteoglycans contribute to provide mechanical and elastic properties to products containing CS. The CS monomer is a

disaccharide compound of N-Acetylgalactosamine and N-glucuronic acid. The sulfate group is fixed in galactosamine, in position 4 and 6, which explains the existence of 2 isomers of CS. [31]

5.3.2.1 Eyeball pharmacokinetics

Route of administration: ophthalmic.

Release: immediate.

Distribution: on the ocular surface and nasal epithelium through the nasolacrimal duct.

Absorption: from the formulation administered topically, it has been determined that there is no absorption through the cornea.

Metabolism: by phase I biotransformation reactions, oxidation and reduction.

Elimination: through the tear system.

5.3.3 Use of conservators

The preservatives are used in order to inhibit microbial growth and suppress biodegradation inside medicines. [32] A great variety of conservatives have been used throughout history. The chronic use of drugs has been linked to irritation and alterations of the ocular surface, it has been established that the conservatives themselves have adverse events, which are attached to those of the active principle that preserve. [33]

The preservatives are classified into detergents and oxidants, and a more recent version of oxidants called buffered by ions. [32] The BAK is the most historically used preservative, belongs to the class of detergents. The reasons for its popularity include its broad spectrum of action and its familiarity with the formulator industry.

Although its effectiveness is well recognized, there are numerous studies documenting the harmful effects of BAK. BAK at concentrations of 0.05-0.1% induces necrosis and concentrations of 0.01% apoptosis. Its effects are cumulative and become more severe at higher concentration and with more frequent exposures. [32] [34] [35] It has been postulated that the presence of BAK is responsible for changes in the conjunctival surface identified by cytology by impression, such as the decrease in the density of goblet cells. [36] [37]

5.4 Justification

Patients who attend SDPL independently of its etiology and degree of severity will have to use ocular lubricants to reduce symptoms and improve their quality of life.

Ocular lubricants are the first line of drugs for eye symptoms related to tear film dysfunction in healthy subjects, with a prevalence of 25 to 35% in people over 60 years of age and 6% in those over 40 years. If we add to this those who mention occasional symptoms or who depend on a work or occupational situation and who use it for intermittent periods throughout their lives, the spectrum of population that will have access to these medications is very extensive. It is estimated that 50% of patients diagnosed with lacrimal film dysfunction without concomitant diseases will use more than 2 ophthalmic solutions in 5 years of treatment.

Taking into account the observation of the cumulative effect of conservatives such as BAK and that their adverse events are directly linked to the amount instilled and their repetitions, it is imperative to provide options that do not have aggravating inflammation secondary to the use of conservatives.

PRO-087 is a preservative-free formulation that requires the documentation of your safety profile.

5.5 Objectives and hypothesis

5.5.1 General Objective

To evaluate the safety and tolerability of the preservative-free ophthalmic solution PRO-087 on the ocular surface.

5.5.2 Specific Objectives

- Describe the safety of the preservative-free ophthalmic solution PRO-087 by means of the density of goblet cells
- Describe the safety of the preservative-free ophthalmic solution PRO-087 by means of changes in intraocular pressure.
- Describe the safety of the preservative-free ophthalmic solution PRO-087 by means of changes in ocular surface stains.
- Describe the safety of the preservative-free ophthalmic solution PRO-087 through changes in laboratory tests.
- Describe the safety of the preservative-free ophthalmic solution PRO-087 by means of changes in vital signs.
- Describe the safety of the preservative-free ophthalmic solution PRO-087 by means of changes in visual capacity.
- Describe the safety of the preservative-free ophthalmic solution PRO-087 by means of changes in the tear-rupture time.
- Describe the safety of the preservative-free ophthalmic solution PRO-087 by means of changes in the ocular comfort index.
- Describe the safety of the preservative-free ophthalmic solution PRO-087 by means of changes in the posterior segment.
- Describe the safety of the preservative-free ophthalmic solution PRO-087 by means of changes in ophthalmological signs and symptoms.
- Describe the safety of the preservative-free ophthalmic solution PRO-087 when assessing the integrity of the anterior segment by means of fluorescein staining.
- Describe the safety of the preservative-free ophthalmic solution PRO-087 when evaluating the integrity of the anterior segment by means of the green lysine stain.
- Describe the safety of the preservative-free ophthalmic solution PRO-087 through the presentation of adverse events.

5.5.3 Hypothesis

Ha The ophthalmic solution PRO-087 presents a safety and tolerability profile similar to comparators in healthy subjects

Ho The ophthalmic solution PRO-087 presents a different safety and tolerability profile compared to comparators in healthy subjects.

5.6 Design and study plan

Clinical trial, phase I, controlled, parallel groups, double blind randomization, exploratory.

5.6.1 Discussion of the study design

The design of the study (clinical trial) is considered the highest quality standard in the data when it is sought to explore the effect of an intervention. The phase of pharmacological development (phase I) corresponds to the objective of the study which is to assess safety and tolerability, so that the intervention time is short and the sample size required is less than that of a clinical efficacy trial. The presence of parallel groups allows the comparison between the intervention groups on the outcome variables. Blinding and randomisation allow to reduce biases that are incurred with other designs, eg. Selection bias, evaluation bias, among others.

6. Material and methods. Participants, interventions and variables

6.1 Center of the study

The present study will be performed in ophthalmology offices duly equipped and registered for their proper functioning. According to the needs of the sponsor, these may be private or public, be attached to a hospital or clinic or be independent.

6.1.1 Organization of the center

Each study center will have a principal investigator (PI). The PI is the ophthalmology specialist in the clinical study.

The PI is responsible for forming a multidisciplinary research team to carry out the clinical study according to protocol, under its scientific guidance. It is the prerogative of the IP the design of the organization of its center and the selection of the personnel that will perform the functions. Nevertheless, the minimum organization of the research team requested by the sponsor requires the figure of sub-researcher, study coordinator and pharmacist. (See **Figure 1**)

Any person to whom the PI designates, under his / her responsibility, a part of the follow-up of the study (co-investigator, under-researcher, nurse, etc.) or a specific function of participation in the study (pharmacist, administrative assistant, study coordinator, etc.) should appear in the "Delegation of Responsibilities" format.

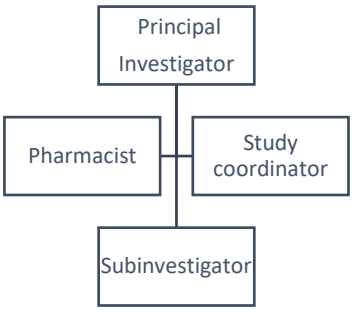


Figure 1 Minimum organization of the center

The "Delegation of Responsibilities" and the "Organizational Chart of the Center" must be delivered to the sponsor before the start of the study and updated if the members or their responsibilities are changed.

6.1.2 Documentation to be delivered to the sponsor

The PI must deliver to the sponsor, before the start of the study:

- Curriculum vitae updated, in Spanish, dated and signed (maximum 10 pages), of the IP and the staff that integrates its organizational chart of the center.

- Copy of IP academic certifications (degree certificate and specialty diploma in ophthalmology, federal professional certificates)

- Copy of academic certifications of the maximum degree obtained, from each one of the members of your research team, that cover their capacity to perform the delegated functions.

- Copy of operation notice or similar issued by corresponding regulatory entity (When applicable)

- Certificate of good clinical practice in force. If the issuing institution does not specify the validity period in the certificate, the date of issue of the certificate must not exceed one year

6.1.3 Closure of the center

The closing of the center will be carried out once the last visit of the last included subject previously agreed between the sponsor and the IP has been made. The closing process will be according to the internal operating procedures of the sponsor.

It is the prerogative of the sponsor to prematurely close a study center, it must inform the IP the reasons for the closure.

6.2 Eligibility criteria

6.2.1 Inclusion criteria

- Signed informed consent.
- Systemically and ophthalmologically healthy subjects evaluated during the clinical history.
- Age between 18 to 40 years.
- Both genders.
- Blood tests [complete blood count (BHc), three-element blood chemistry (QS) and liver function tests (PFH)] within normal parameters specified by the reference laboratory with a lower and upper margin of 10%.

- Vital signs within normal parameters.
- Visual ability 20/30 or better, in both eyes.
- Intraocular pressure ≥11 and ≤ 21 mmHg.

6.2.2 Exclusion criteria

6.2.2.1 General criteria

- Subjects with a history of hypersensitivity to any of the components of the research products.
- Subject users of topical ophthalmic medications of any pharmacological group.
- Subject users of medication by any other route of administration.
- Pregnant or lactating women.
- Women without a history of hysterectomy, oophorectomy or hysterectomy, who do not ensure a hormonal contraceptive method or intrauterine device during the study period.
- Subjects with participation in clinical research studies 90 days prior to inclusion in the present study.
- Diagnosis of liver disease or triple the normal upper value of any of the following liver enzymes: aspartate transferase (AST), alanine transferase (ALT) or bilirubin.
- Inability to attend or answer the evaluations made in each of the visits.
- Positive tobacco use (specified as cigarette consumption regardless of quantity and frequency)
- Positive alcoholism (specified as the consumption of alcoholic beverages, regardless of quantity and frequency, during the study intervention period).
- Contact lens users.

6.2.2.2 Medical and therapeutic criteria

6.2.3 Elimination criteria

- Withdrawal of the consent letter under information.
- Presentation of serious adverse event.
- No tolerability or hypersensitivity to any of the compounds used during the tests (fluorescein, green lysine, tetracaine)
- No tolerability or hypersensitivity to any of the investigational drugs.
- Adherence <50% determined by the diary of the subject or by the weight of the bottle, of the established dose of the pharmacological intervention in any of the visits.

6.2.4 Identification of the subject

The patients of the study 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 maximum three letters, in case the person has two names or a compound surname the first letter will always be used.

Example:

1. Arieh Daniel Mercado Carrizalez

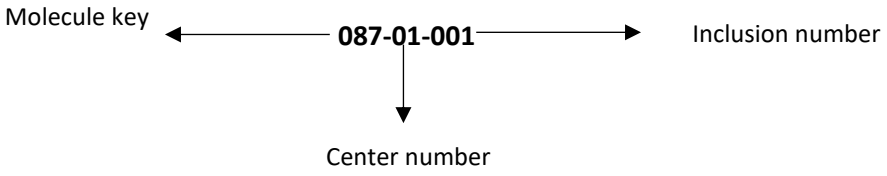
a. Initials: AMC

2. Juan De la Torre Orozco
   a. Initials: JDO

In the counting stage, the participant number will be assigned consecutively, using 3 consecutive digits. Once the subject has been selected, he will be assigned a number with which he will be identified throughout the study. Said code will be composed of eight numbers in the following order from left to right:

- Three digits of the molecule under study according to the denomination by the sponsor.
- Two digits corresponding to the research center number.
- Three digits of the number consecutive to its inclusion assigned to the research center.

Example:



6.3 Intervention

6.3.1 Managed treatments

6.3.1.1 Treatment in study.

- **PRO-087**
  - o Active ingredients (lubricant): chondroitin sulfate 0.18%, sodium hyaluronate 0.1%
  - o Pharmaceutical form:
  - o Prepared by: Sophia Laboratories, S.A. of C.V.
  - o Dosage: 1 drop in both eyes, 4 times a day during the waking period
  - o Description of the solution: transparent solution, free of visible particles.
  - o Description of container: sterile multi-dose bottle

Table 4. Quali-quantitative formulation of PRO-087

Type of agent	Quantity mg/mL	Function
Chondroitin sulfate	1.8	Active principle (lubricant)
Sodium hyaluronate	1	Active principle (lubricant)
Boric acid	2.00	Active principle
Sodium borate decahydrate	Without showing	Additive
Polysorbate 80	Without showing	Additive
Sorbitol	Without showing	Additive
Potassium chloride	Without showing	Additive
Sodium chloride	Without showing	Additive

Type of agent	Quantity mg/mL	Function
Magnesium chloride hexahydrate	Without showing	Additive
Water for the preparation of injectables c.b.p.	1.00	Vehicle

Quali-quantitative formulation of the product under investigation PRO-087. The concentration of the active principles is shown, as well as the substances that act as a buffer additive.

6.3.1.2 Reference treatment

- **Xyel Ofteno®**
  - Active principles:
  - Pharmaceutical form: Ophthalmic solution
  - Prepared by: Sophia Laboratories, S.A. of C.V.
  - Dosage: 1 drop in both eyes, 4 times a day during the waking period
  - Description of the solution: transparent solution, free of visible particles.
  - Description of container: sterile multi-dose bottle
- **Systane Ultra®**
  - Active ingredients:): Polyethylene glycol 400 0.4%, propylene glycol 0.3%
  - Pharmaceutical form: Ophthalmic solution
  - Presentation: multi-dose dropper bottle
  - Prepared by: Alcon Laboratories, Inc.
  - Dosage: 1 drop in both eyes, 4 times a day during the waking period.

6.3.2 Strategies to improve adherence and procedure to monitor adherence

1. Each visit the research subject will return the assigned bottle in order to evaluate the adherence by weight.
2. Direct questioning by the IP about the application of the intervention.
3. At the IP criteria, messages can be sent or reminder calls can be made.
4. Delivery of printed chronogram specifying the date of the visit and its activities
5. Journal of the subject.

6.3.2.1 Procedure to monitor adherence

Before delivering the medication to the research subject, the pharmacist must carry out the weighing of the bottles to be delivered. After the return of the medication by the subject will also carry out the weighing. Guidelines to follow for weighing:

- The pharmacist will use the scale provided by the sponsor
- Place the bottle in the center of the scale. You will get the result of the measurement.
- Remove the jar from the balance and put it back in, confirming that the measurement is the same. If it is different, it will weigh one more time and take the average of the 3 measurements
- Record the result in the log and the CRF provided by the sponsor

The adhesion will be calculated considering: the weight of the empty bottle, the weight of the drop, the weight of the bottle with the content, the calculation of the total of drops to be applied during the entire time of intervention and the total weight of the drops applied. The following simplified formula will be used:

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

Where:
Ad = Adhesion
P_i = weight of the bottle delivered to the subject at the start
P_f = weight of the bottle returned by the subject
P_T = weight of the posology indicated for the intervention

$$P_T = (P_g)G$$

Where:
P_g = weight of the intervention drop, determined by the research and development department
G = number of drops indicated for the intervention

Adherence will be estimated at each visit where the research subject returns the intervention. Only those bottles without apparent physical damage will be considered for the calculation. This result will allow the PI to determine if the subject continues in the study according to the stipulations of the elimination criteria.

The evaluation of the adherence by means of the diary of the subject will be carried out in the following way:

$$Ad = (A_r)100/A_i$$

Ad = Adherence
A_r = Registered applications
A_i = Applications indicated for the intervention

The final (overall) adhesion will be determined by the average of the adherence of each of the visits.

It will not be considered for the calculation of final adherence if the subject did not return the bottle in two subsequent visits.

6.3.3 Treatments and concomitant interventions allowed and prohibited during the study.

The use of concomitant medications by any route of administration during the intervention period will not be allowed. Except those specified for the study procedures. The objective of this restriction is to avoid pharmacological interactions that could alter the results of the evaluated variables.

6.3.4 Treatment management.

The interventions will be provided by Sophia Laboratories, S.A. of C.V., for each research center. They will be labeled, reconciled and weighed previously. The handling of the treatment will be under the responsibility of the researcher or a designated member of his team.

6.3.4.1 Delivery and reception.

Delivery will be made in closed cardboard boxes by means of a courier service or directly by the sponsor's staff to the home of the research center according to the study plan.

The reception will be exclusively carried out by the research center team, including the researcher. You must check the good condition of the primary packaging (box). In the event that it shows alterations or defects in its integrity that from its judgment could have damaged the content, it should report it to the sponsor. If the package does not show significant defects, it will proceed to open it.

Inside you must locate the acknowledgment document and the logger (data logger) of temperature and humidity. You should check that the registered temperature and humidity comply with the specifications for transport and shelter (see section 6.3.4.2 Storage). Verify the content (interventions) with what is reported in the document. In case the document corresponds to the content, it will sign the receipt and send it to the sponsor. Otherwise, notify the sponsor.

In the study center, the personnel assigned by the PI will deliver the corresponding treatment to the subjects admitted, sufficient for the period to be covered. The delivery will be made in stages, medication will be delivered at the baseline visit, on visits 1 and 2. The center must register the medication delivered.

6.3.4.2 Storage.

The medication must be stored in a secure area with restricted access.
The storage temperature should be <30 ° Celsius.
The research center has the obligation to record, in the format designated by the sponsor, the temperature and humidity registered in the data logger. This record should include the current temperature and humidity, as well as the minimum and maximum of each of these. It must be done at least once a day, on business days.
Said data will be compared by the clinical monitor according to the registration in the data logger.

6.3.4.3 Return.

The research subjects will return to the personnel indicated by the IP in the center their treatments in visits 1, 2 and final. The refund will be made by the research center when the sponsor indicates it. Prior to the return the research center must make a count of the assigned medication and the remaining medication, with the aim of creating an inventory which serves for the final filling of the medication return form.

6.4 Outcome variables.

6.4.1 Security variables.

6.4.1.1 Primary outcome variables.

- Density of goblet cells
- Presence of adverse events.
- Intraocular pressure.
- Visual ability
- Laboratory tests: BHc, QS and PFH.
- Epithelial defects in cornea and conjunctiva.
- Ophthalmological signs: conjunctival hyperemia, chemosis.

6.4.1.2 Primary outcome variables of tolerability.

- Burning
- Foreign body sensation

-Itching

-Eye comfort index

6.4.1.3 Secondary outcome variables.

- Rupture time of the tear film
- Life signs: FC, FR, TAS.
- Subsequent segment
- Quality questionnaire.

6.4.2 Efficacy variables.

6.4.2.1 Primary outcome variables.

Does not apply because it is a phase I study.

6.4.2.2 Secondary outcome variables.

Does not apply because it is a phase I study.

6.4.3 Methods and scales to be used for the measurement of the variables

Variable	Unity	Symbol	Type	Method of measurement	Normal value
Age	Years	--	Continuous	Calculation from the date of birth	NA
Gender	Female Male	F / M	Nominal	Direct questioning	NA
Adverse events	Number of cases	n	Discreet	Count	NA
Intraocular pressure	Milimeters of mercury	mmHg	Continuous	Goldman´s applanation tonometry	11 - 21
Visual ability	Fraction	Snellen	Nominal	Primer	
Tear rupture time	Seconds	s	Continuous	Direct count	> 10
Eye comfort index	points	--	Discreet	Questionnaire	
Adverse events	Present / Absent	--	Nominal	Comprehensive valuation	Absent
Goblet cell density	Cells per square millimeter	Cel/mm^2	Continuous	Cytology by impression	> 500 cel/mm^2
Vital signs					
Heart rate	Beats per minute	lpm	Discreet	Auscultation	60 – 100
Breathing frequency	Breaths per minute	rpm	Discreet	Auscultation	12 – 24
Systemic blood pressure	Milimeters of mercury	mmHg	Continuous	Non-invasive auscultatory measurement	< 120 / 80
Previous segment					
Epithelial defects	Degrees	--	Discreet	Direct observation with fluorescein and green lysamin stain	Oxford Scale

Variable	Unity	Symbol	Type	Method of measurement	Normal value
Ophthalmologic signs and symptoms					
Conjunctival hyperemia	Normal / Very Light / Mild / Moderate / Severe	--	Ordinal	Direct observation. Classification of Efron.	Normal
Chemosis	Present / Absent	--	Nominal	Direct observation	Absent
Burning	Severity: Absent, very mild, mild, moderate and severe	--	Nominal	Direct questioning	Absent
Foreign body sensation	Severity: Absent, very mild, mild, moderate and severe	--	Nominal	Direct questioning	Absent
Pruritus	Frequency: At all times, almost at all times, 50% of the time, almost in no time, at any time	--	Nominal	Direct questioning	Absent
Postinstilation symptoms					
Burning	Present / Absent	--	Nominal	Journal of the subject	Absent
Foreign body sensation	Present / Absent	--	Nominal	Journal of the subject	Absent
Pruritus	Present / Absent	--	Nominal	Journal of the subject	Absent
Red eye (symptom)	Present / Away	--	Nominal	Journal of the subject	Absent

Variable	Unity	Symbol	Type	Method of measurement	Normal value
Posterior segment					
Macula	Normal / Abnormal	--	Nominal	Observación directa	Normal
Optical disk integrity	Normal / Abnormal	--	Nominal	Observación directa	Normal
Blood count					
Erythrocytes		M/uL	Continuous		
Hemoglobin	Grams over deciliter	g/dL	Continuous		
Hematocrit	Percentage	%	Continuous		
VGM	Femto liters	fL	Continuous		
HCM	picograms	pg	Continuous		
CMHbG	Grams over deciliter	g/dL	Continuous		
Leukocytes	Thousands per liter units	Mil/uL	Continuous		
Platelets	Thousands per liter units	Mil/uL	Continuous		
Myelocytes	Percentage	%	Discreet		
Metamyelocytes	Percentage	%	Discreet		
Bands	Percentage	%	Discreet		
Segmented	Percentage	%	Discreet		
Lymphocytes	Percentage	%	Discreet		
Monocytes	Percentage	%	Discreet		
Eosinophils	Percentage	%	Discreet		
Basophils	Percentage	%	Discreet		
Blastos	Percentage	%	Discreet		
Blood chemistry					
Glucose	Milligrams on deciliter	mg/dL	Continuous		
Urea	Milligrams on deciliter	mg/dL	Continuous		
Creatinine	Milligrams on deciliter	mg/dL	Continuous		
Liver function tests					

Variable	Unity	Symbol	Type	Method of measurement	Normal value
Alanine transferase	Units on liter	U/L	Continuous		
Aspartate transferase	Units on liter	U/L	Continuous		
Total bilirubin	Milligrams on deciliter	mg/dL	Continuous		
Direct bilirubin	Milligrams on deciliter	mg/dL	Continuous		
Indirect Bilirubin	Milligrams on deciliter	mg/dL	Continuous		

Table 5. Scales to be used.

The following describes the methods and scales that will be used for the measurement of the variables, which are in strict alphabetical order:

6.4.3.1 Visual ability.

Visual acuity (VA) is a test of visual function. Spatial visual acuity is the ability to distinguish separate elements of an object and identify them as a whole. It is quantified as the minimum separation angle (located at the nodal point of the eye) between two objects that allows perceiving them 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) equals 5 minutes of arc, a letter 6/12 (20/40) equals 10 minutes, and a letter 6/60 (20/200) equals 50 minutes The Snellen fraction can also be expressed as a decimal (ie 20/20 = 1 and 20/40 = 0.5). [38]

The VA will be evaluated basally, without refractive correction with the Snellen chart. Which will be located in a place with adequate lighting, natural or artificial and at a distance of 3m from the subject to be evaluated. The visual acuity of each eye will be taken, starting with a right eye (DO) asking the subject to keep both eyes open and using an occluder to cover the left eye (OS); the subject will read aloud the lines that the evaluator points out, the line of smaller letters that he reaches to see will be annotated by the fractional evaluator as the DO of the DO in the clinical record. Proceed to the OS with the same method.

Subsequently the best refractive correction of the subject will be made and the examination will be repeated using the obtained refraction. This result will be reported as CV, it will be written in fraction in the clinical file and in the CRF, in addition in the CRF it will be written in decimal. By definition, the CV can not be inferior to the AV.

6.4.3.2 Goblet cell density (Cytology per impression).

The density of goblet cells in the conjunctiva can be a reflection of the severity of alteration in the ocular surface. [39] It has been determined that the normal density is greater than 500 cel / mm2. [40] Impression cytology refers to the application of a cellulose acetate filter to the ocular surface to remove the superficial layers of the epithelium; these removed cells can be subjected to histological, immunohistological or molecular analysis. [41]

The cytology per impression of the conjunctiva will be done by the researcher using the device provided by the sponsor for this purpose, which may consist of a circular cell of approximately 10 mm in diameter, with a cellulose acetate filter millicelll. After ocular surface anesthesia, with topical

tetracaine, the researcher will ask the subject to expose the temporal surface of the eye to be evaluated and gently press the cell on the temporal conjunctiva (2 to 3 mm of the sclerocorneal limbus) for 5 seconds and remove the cell with a peeling technique (Illustration 1. Cytology per impression). Immediately fix with diethyl ether spray with one or two shots at 15 cm distance. The investigator will contact the Courier designated by the sponsor for the delivery of the samples to the histopathological analysis.

The data of the histopathological report to be recorded in the CRF are: density of goblet cells and degree of squamous metaplasia according to the Nelson scale. (**See Table 6**)

In the visits that are required to perform this procedure, it will be done prior to the use of ocular surface stains.

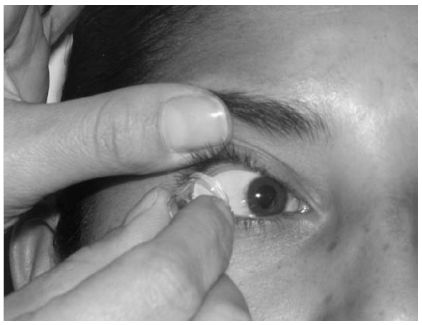


Illustration 1. Cytology per impression.

Table 6. Nelson classification

Grade	Detail description of the epithelial cells	Characteristics of goblet cells
0	Small and round cells; Large nuclei, 1: 2 ratio with nuclear cytoplasm	Abundant goblet cells (> 500 cells / mm2), globose and oval with an intense PAS-positive staining of the cytoplasm.
1	Slightly enlarged cells; smaller nuclei, 1: 3 ratio with nuclear cytoplasm	Marked decrease in the number of goblet cells (350 to 500 cells / mm2) but still maintain their globose and oval shape and intense staining
2	Enlarged and polygonal cells; small nuclei, ratio 1: 4 to 1: 5 with nuclear cytoplasm	Marked decrease in the number of goblet cells (100 to 350 cells / mm2), less intense PAS - positive with poorly defined cell borders
3	Even larger and polygonal cells; small and pyknotic nuclei, 1: 6 ratio with nuclear cytoplasm	Few goblet cells (<100 cells / mm2)

Adapted from Nelson & Wright [24] [42]

6.4.3.3 Eye comfort index.

It is a questionnaire designed to measure the irritation of the ocular surface with Rasch analysis to produce estimates on a linear scale of intervals (ratings: 0-100). Similar to the index for ocular

surface diseases, the ocular comfort index (ICO) evaluates symptoms. The ICO contains 8 items (one positive and eight negative) that focus on the discomfort associated with alterations of the ocular surface. Each of these questions has two parts, which inquire separately the frequency and severity of the symptoms. [43] See annex 13.1 Eye comfort index.

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

6.4.3.4 Eye surface integrity:

This will be done by means of biomicroscopy using the slit lamp of the research center. A full assessment of the previous segment will be made, which will be recorded in the clinical file. The lighting techniques used will be at the discretion of the IP.

The variables that will be registered in this protocol are:

- *Conjunctival hyperemia.*
  It is defined as the simplest reaction of the conjunctiva to a stimulus, a red appearance secondary to the vasodilation of the conjunctival vessels of variable intensity. He will graduate using the Efron scale. [44] See annex 13.2 Efron scale for conjunctival hyperemia
- *Chemosis.*
  It is defined as conjunctival edema, the result of an inflammatory reaction. It is qualified as present or absent. The evaluator will use a narrow beam of light at 60 ° and will measure if the conjunctiva separates from the sclera at ≥ 1/3 of the total palpebral opening or if it exceeds the gray line. [45]

6.4.3.4.1 Stains.

- *Staining with green lysine.*

  A drop of topical anesthetic will be instilled in the conjunctival cul-de-sac, then a second drop will be applied to the tip of the strip of green lysine and it will be allowed to slip towards the bottom of the sac. It is essential to quickly evaluate the staining, in sequence, first in the DO and then the OS, so that the observed patterns are equally bright. [19] See Annex 13.3 Oxford Scale

- *Fluorescein staining.*

  A drop of topical anesthetic will be instilled into the conjunctival cul-de-sac, then a second drop will be applied to the tip of the fluorescein strip and it will be allowed to slip to the bottom of the sac. It is essential to quickly evaluate the staining, in sequence, first in the DO and then the OS, so that the observed patterns are equally bright. This valuation will be made with the cobalt blue filter. [19] See Annex 13.3 Oxford Scale

For both stains, the value obtained according to the Oxford scale will be registered in the CRF.

6.4.3.5 Presence of adverse events.

The management of the EAs will be done according to what is described in section 9.3 Adverse events

The PI will register in the corresponding section of the CRF the EAs that come to present the subjects of the study in addition to referring it in its essential document.

6.4.3.6 Intraocular pressure.

Tonometry is the objective measure of IOP, based primarily on the force required to flatten the cornea or the degree of corneal indentation produced by a fixed force. Goldman's tonometry is based on the Imbert-Fick principle. [38] The tonometry will be performed, after instillation of a drop of topical anesthetic (tetracaine 0.5%), with fluorescein and the use of the cobalt blue filter (after evaluation of the corneal surface staining). There will be 3 shots, which will be recorded in the clinical file and the average will be registered in the CRF.

6.4.3.7 Posterior segment.

The evaluation of the posterior segment will be carried out under medication mydriasis (tropicamide 0.8% / phenylephrine 5%), in the slit lamp with an aerial loupe (at the choice of the PI). An integral assessment of the fundus (including optic disk, posterior pole and periphery) will be performed in search of abnormalities that alter the study result. The result of the assessment will be recorded in the clinical file. The CRF will record the assessment of the macula and optic nerve as normal, abnormal or abnormality that does not affect.

6.4.3.8 Vital signs.

The vital signs to be evaluated (FC, FR and TAS) can be measured by an assistant duly indicated in the organization of the center and the delegation of responsibilities, the technique to be used for the FC and FR will be with the count of repetitions in one minute by Direct auscultation with stethoscope.

The SBP should be measured with 5 minutes of previous rest, in the left arm. The instrument can be manual or automatic according to the IP. It is necessary that all measurements are equal in circumstances. 3 measurements will be made, with a minimum interval of 5 minutes between them. The IP will record the average in the note and the CRF.

6.4.3.9 Ocular symptomatology.

The subject will be questioned directly about the presence in general (since the last visit) of the following symptoms: burning, foreign body sensation and pruritus. Respond about the severity and frequency of symptoms such as:

Severity: Absent (0), very mild (1), mild (2), moderate (3) and severe (4)

Frequency: At all times (4), almost at all times (3), 50% of the time (2), almost at no time (1), at any time (0).

The number corresponding to each symptom will be registered in the CRF.

Symptomatology postinstilación:

The subject will be requested, that in the subject's daily instrument, after registering the application of the product under investigation, mark YES or NO according to the presence of the following symptoms: burning, sensation of a foreign body, red eye and pruritus.

6.4.3.10 Breaking time of the tear film.

One of the first aspects of the tear film that changes when there is an alteration to the ocular surface, is its stability. In general, if the corneal or conjunctival surface is damaged, it is unlikely that a stable tear film can be maintained.

The most common method to evaluate the stability of the tear film is the evaluation of TRL with fluorescein. Once the fluorescein is instilled, with the cobalt blue filter the patient is asked not to blink. The precorneal colored fluorescein layer will change to less fluorescent or non-fluorescent

regions. The time that elapses from the last blink until the appearance of these regions is the TRL. It will be reported in seconds, in the clinical file and in the CRF.

6.4.3.11 Pregnancy test.

It refers to the performance of a rapid pregnancy test in all women of childbearing age who wish to enter the study. By fertile age we understand women who have not had their menopause, defined as 12 months since the last menstrual period in women over 40 years of age; or those who underwent bilateral hysterectomy or oophorectomy. Women of childbearing age with contraceptive methods including bilateral tubal obstruction should be tested for pregnancy. This test will be carried out by the IP or the designated team person according to the instructions of the device delivered by the sponsor. When applicable, the completion, result and date must be registered in the CRF. If you do not apply, you must write down the reason.

6.4.3.12 Lab tests.

The PI will deliver to the subject the order of the studies of BH, QS and PFH, to be carried out in the clinical laboratory designated by the sponsor. The clinical laboratory will deliver to the IP the results for its assessment and registration. The normal parameters to be considered will be the ranges established by the laboratory, Nevertheless the clinical criterion of the PI will prevail in the decision of normality or abnormality of the results.

6.4.4 Measurement time.

The measurements of the variables of primary and secondary outcome will be made and evaluated for each visit, according to the following:

Basal Visit / Day 0.

Some of these measurements will be taken at the screening visit to complete the eligibility criteria (see Schedule and study diagram), at the discretion of the PI, they may be taken to complete the data of the baseline visit.

1. Visual ability
2. Density of goblet cells (Cytology per impression)
3. Intraocular pressure.
4. Eye comfort index
5. Evaluation of ocular signs and symptoms
6. Integrity of ocular surface
    a. Includes stains
    b. TRL
7. Subsequent segment evaluation.
8. Vital signs
9. Evaluation of results of laboratory tests.

Visit 1 / Day 3.

It can be done in a period ± 1 day in relation to day 3 of application.

1. Visual ability
2. Intraocular pressure.
3. Evaluation of ocular signs and symptoms
4. Integrity of ocular surface
    a. Includes stains
    b. TRL
5. Vital signs
6. Evaluation of adverse events.

Visit 2 / Day 6.
It can be done in a period ± 1 day in relation to the 6th day of application.

1. Visual ability
2. Intraocular pressure.
3. Evaluation of ocular signs and symptoms
4. Integrity of ocular surface
   a. Includes stains
   b. TRL
5. Vital signs
6. Evaluation of adverse events.

Final Visit / Day 11.
It can be done in a period ± 1 day in relation to the 11th day of the start of application.

1. Visual ability
2. Density of goblet cells (Cytology per impression)
3. Intraocular pressure.
4. Eye comfort index
5. Evaluation of ocular signs and symptoms
6. Integrity of ocular surface
   a. Includes stains
   b. TRL
7. Subsequent segment evaluation.
8. Vital signs
9. Evaluation of adverse events.

Security call / Day 13.
It can be done in a period ± 1 day in relation to the 13th day of the start of application.

1. Ask about the presence of an adverse event.
2. Evaluation of results of laboratory tests

6.5 6.5 Timeline and study diagram.

Procedures	*Scrutiny*	Basal Visit Day 0	Visit 1 Day 3 ± 1	Visit 2 Day 6 ± 1	Final Visit Day 11 ± 1	Call Security Day 13 ± 1
CI Signature	X					
Clinic history	X					
Ophthalmological clinical history	X					
Laboratory sample taking	X				X	
Laboratory tests review		X				X
Pregnancy test	X				X	
Eligibility criteria	X	X[a]				
Assignment		X				
Delivery of intervention		X	X	X		
Return of intervention			X	X	X	
Adherence evaluation			X	X	X	
Adverse events			X	X	X	X
Intraocular pressure	X	X[1]	X	X	X	
Visual ability	X	X[1]	X	X	X	
TRL	X	X[1]	X	X	X	
Epithelial defects (TF and TVL)	X	X[1]	X	X	X	
Eye signs and symptoms	X	X[1]	X	X	X	
posterior segment	X	X[1]			X	
Vital signs	X	X[1]	X	X	X	
Ocular Comfort Index		X			X	
Goblet cell density (cytology by impression)		X			X	
Daily delivery of the subject		X	X	X		
Return / Evaluation of the subject's Journal			X	X	X	
Delivery Quality Questionnaire		X				
Return and Evaluation of the quality questionnaire					X	
Continuity evaluation of the subject			X	X		

[a] The eligibility criteria will be completed with the revision of the laboratory exams. 1 These measurements may be taken from the result of the screening visit, if it does not exceed the previous 7 days. It is the prerogative of the PI to decide whether to repeat the measurements at the baseline visit.

6.5.1 Procedures to be performed per visit.

6.5.1.1 Scrutiny visit.

- Signature of informed consent: refers to the signing of the written informed consent document. See 10.3 Consent (assent)
- General and ophthalmological clinical history: refers to the technical, clinical and legal document in which the patient's health conditions, medical acts and other procedures performed on the patient are recorded chronologically. It includes the anamnesis and comprehensive ophthalmological exploration that allows to discern the patient's eligibility. If the patient is taken from the established consultation of the study center, he / she will be able to use the existing clinical history, only having to perform an update.
- Taking laboratory samples: see 6.4.3.12 Laboratory tests.
- Pregnancy test: see 6.4.3.11 Pregnancy test.
- Eligibility criteria: refers to the review by the IP, where it states that the subject can be included in the study by meeting the inclusion criteria and not meeting the exclusion criteria. See 6.2 Eligibility criteria
- Intraocular pressure: see 6.4.3.6 Intraocular pressure
- Visual ability: see 6.4.3.1 Visual capacity
- TRL: see 6.4.3.10 Rupture time of the tear film
- Epithelial defects (TF and TVL): see 6.4.3.4.1 Stains
- Eye signs and symptoms: see 6.4.3.4 Eye surface integrity: and 6.4.3.9 Ocular symptomatology
- Subsequent segment; see 6.4.3.7 Subsequent segment
- Vital signs: see 6.4.3.8 Vital signs

6.5.1.2 Basal Visit .

- Review of laboratory tests: refers to the review and analysis by the IP of the results of the BH, QS and PFH. See 6.4.3.12 Laboratory tests.
- Eligibility criteria: with the laboratory results, the subject's profile will be finalized for inclusion or not.
- Assignment: It refers to determining the intervention that the patient will follow during the study. It will be done according to section 7. Methods. Assignment of the intervention. This assignment will be made at the baseline visit (day 0) and will go along with the indication to start the treatment period the next day (day 1).
- Delivery of intervention: Refers to the delivery of the product under investigation to the patient of the study, by the research center. It will be done according to sections 6.3.1 Managed treatments and 6.3.4.1 Delivery and reception.
- Evaluation of variables: The data of the evaluation of the variables listed below can be taken from the scrutiny visit, as long as it does not exceed 7 days prior to this visit. It is the prerogative of the IP to decide whether to use the information from the screening visit or to repeat the evaluations in this visit.
    - Intraocular pressure
    - Visual capacity
    - TRL
    - Epithelial defects
    - Eye signs and symptoms

- Subsequent segment
- vital signs

- Eye comfort index: see 6.4.3.3 Eye comfort index
- Density of goblet cells (cytology per impression): see 6.4.3.2 Density of goblet cells (cytology per impression)
- Delivery of the subject's diary: It refers to the delivery by the IP to the subject, the subject's daily instrument.
- Delivery of quality questionnaire: It refers to the delivery by the IP to the subject, the quality questionnaire instrument.

6.5.1.3 Visit 1.

- Intervention delivery: see 6.5.1.2 Baseline visit
- Return of intervention: refers to the return of research products to the center by the research subject.
- Evaluation of adherence: refers to the assessment made by the IP according to section 6.3.2.1 Procedure to monitor adherence
- Adverse events: see 6.4.3.5 Presence of adverse events
- Intraocular pressure: see 6.4.3.6 Intraocular pressure
- Visual ability: see 6.4.3.1 Visual capacity
- TRL: see 6.4.3.10 Rupture time of the tear film
- Epithelial defects (TF and TVL): see 6.4.3.4.1 Stains
- Eye signs and symptoms: see 6.4.3.4 Eye surface integrity: and 6.4.3.9 Ocular symptomatology
- Vital signs: see 6.4.3.8 Vital signs
- Submission of the subject's diary: see 6.5.1.2 Baseline visit
- Return / daily evaluation of the subject: refers to the delivery of the subject's diary to the IP by the subject. The PI will review the diary to assess its correct filler, evaluate postinstilation symptoms and record applications.
- Continuity assessment of the subject: refers to the determination by the IP and desire of the subject to continue with their participation in the study.

6.5.1.4 Visit 2

- Intervention delivery: see 6.5.1.2 Baseline visit
- Return of intervention: see 6.5.1.3 Visit 1.
- Evaluation of the adherence: it refers to the valuation made by the IP according to the section
- Adverse events: see 6.4.3.5 Presence of adverse events
- Intraocular pressure: see 6.4.3.6 Intraocular pressure
- Visual ability: see 6.4.3.1 Visual capacity
- TRL: see 6.4.3.10 Rupture time of the tear film
- Epithelial defects (TF and TVL): see 6.4.3.4.1 Stains
- Eye signs and symptoms: see 6.4.3.4 Eye surface integrity: and 6.4.3.9 Ocular symptomatology
- Vital signs: see 6.4.3.8 Vital signs
- Submission of the subject's diary: see 6.5.1.2 Baseline visit
- Return / daily evaluation of the subject: see 6.5.1.3 Visit 1.
- Subject's continuity assessment: see 6.5.1.3 Visit 1.

6.5.1.5 Final Visit

- Laboratory sample taking: ver 6.4.3.12 Laboratory tests

- Pregnancy test: see Pregnancy test
- Return of intervention: see 6.5.1.3 Visit 1.
- Evaluation of the adherence: it refers to the valuation made by the IP according to the section
- Adverse events: see 6.4.3.5 Presence of adverse events
- Intraocular pressure: see 6.4.3.6 Intraocular pressure
- Visual ability: see 6.4.3.1 Visual capacity
- TRL: see 6.4.3.10 Rupture time of the tear film
- Epithelial defects (TF and TVL): see 6.4.3.4.1 Stains
- Eye signs and symptoms: see 6.4.3.4 Eye surface integrity: and 6.4.3.9 Ocular symptomatology
- Subsequent segment; see 6.4.3.7 Subsequent segment
- Vital signs: see 6.4.3.8 Vital signs
- Eye comfort index: see 6.4.3.3 Eye comfort index
- Density of goblet cells (cytology per impression): see 6.4.3.2 Goblet cell density (Cytology per impression)
- Return / daily evaluation of the subject: see 6.5.1.3 Visit 1.
- Return / evaluation of the quality questionnaire: refers to the delivery of the quality questionnaire to the IP by the subject.

6.5.1.6 Security call.

- Adverse events: see 6.4.3.5 Presence of adverse events
- Review of laboratory tests: see 6.5.1.2 Baseline visit .

6.5.2 Diagram of the study.

An enrollment time of 30 days is estimated for the total sample.

In order to increase the safety of the participants with the use of the products under investigation, a partial analysis will be carried out following a modification to the Fibonacci method. Said methodology will consist of including 33% of the total sample size (3 subjects per group, n = 9), which will complete all the visits and procedures described. Once this group of 9 subjects has finished their participation in the clinical study, they will proceed to perform a blinded analysis. During the period in which this sub-analysis is carried out, the inclusion of new participants will be restricted. Inclusion will resume when the sponsor notifies each research center.

In addition to safety, the therapeutic adherence will be evaluated and problems related to the procedures will be solved, should they arise.

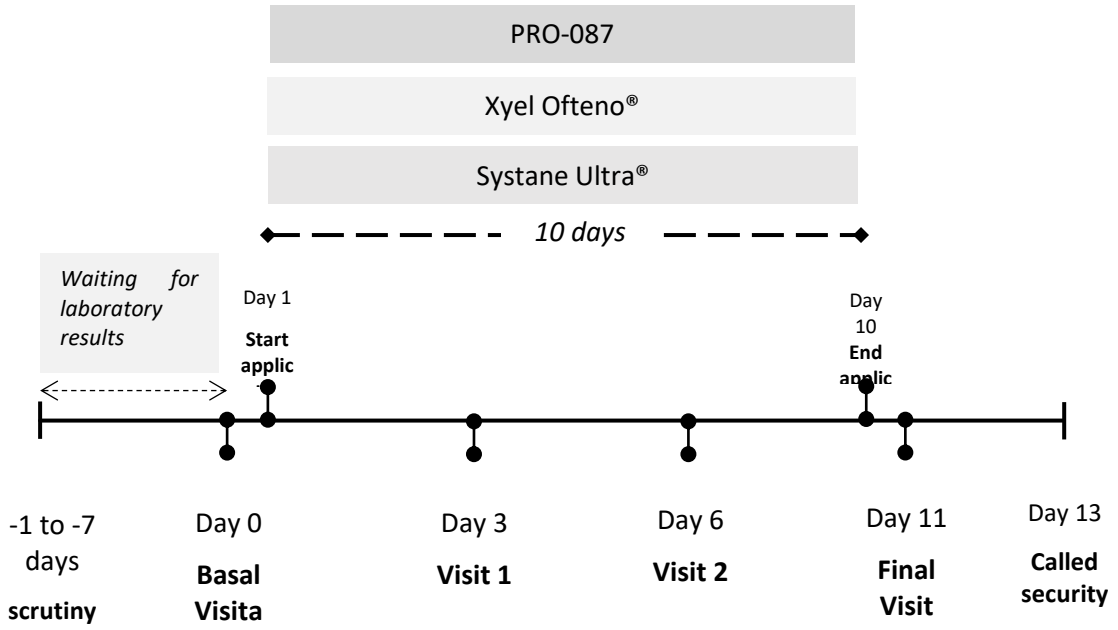


Figure 3. Diagram of the study.

6.6 Sample size.

A total size of 30 subjects is estimated, divided into 3 intervention groups. (10 subjects per group)

6.6.1 Calculation of the sample size.

Although there are no references on the calculation of sample size in phase I studies, it was considered pertinent to perform it according to the presentation of adverse events reported by Hwang HS, et al, in a case-control study in 150 subjects with ophthalmic solutions with Hyaluronate sodium and diquafosol with and without preservative for 3 months. [46].

The percentage of adverse events that occurred with the preservative-free HS solution was 8%, which is why we consider that at least more than 60% of the exposed subjects will not present this rate of adverse events.

The sample size was calculated using the formula for proportions

$$n = \left[(p1)(p2) + (p1)(p2)\left(\frac{Z_{1-\alpha}}{2} + Z_{1-\beta}\right)^2\right]/(p1 - p2)^2$$

With a statistical confidence of 95% corresponding to the type I error, equal to 1.96, with a power of 80%, corresponding to the type II error, equal 0.84.

According to the previous calculation, the result is 7.6 subjects (8) per group. The total when considering 3 intervention groups is 24 subjects, which was increased by 25% due to the probable losses. The total sample size required is 30 subjects. Therefore, each group will consist of 10 subjects, who will provide both eyes for the analysis, so that the total sample to be analyzed will be composed of 60 eyes.

6.7 Recruitment

It is recommended that during the development of this research protocol, the principal investigator requests the approval of the Research Ethics Committee and the Research Committee, as well as the authorization to the relevant regulatory entity, to publish or disseminate in the mass media, the invitation to participate in the study to those people who meet the eligibility criteria.

It is possible to discuss with other health professionals the opportunity for healthy subjects to be evaluated by an ophthalmologist at no cost, as well as cabinet exams that will allow the more accurate determination of their ocular clinical status by participating in a sponsored clinical research protocol. by Sophia Laboratories, SA of C.V.

7. Methods Assignment of the intervention

7.1 Generation of the allocation sequence

The random numbers will be generated using the online tool

: www.randomization.com

3 strata corresponding to the intervention groups will be used, which will be balanced for a research center. The allocation will be 1: 1: 1.

7.2 Blinding mechanism

Blinding will be performed by personnel assigned by the Clinical Operations Management of Sophia Laboratories, S.A de C.V. This will consist of the elimination of the primary label (commercial) in the case of Xyel Ofteno® and Systane Ultra® and the placement of a label identical to the other interventions. Because the bottle in which Systane Ultra® is packaged differs in the color and shape of the lid used by Xyel Ofteno® and PRO-087, a masking will be carried out on the primary packaging which will be identical for the three interventions.

7.3 Implementation

The allocation sequence will be generated by personnel assigned by the Clinical Operations Management of Sophia Laboratories, S.A. of C.V.The research center will receive a set of envelopes which will contain the intervention number individually. The envelopes will be identical on the

outside. Each of these envelopes will be shown to the participants for their election by the principal investigator or by a designated member of their team.

7.4 Blinding (Masking)

The blinding will correspond to the research subject and the principal investigator. In addition, the statistical analysis will be carried out in a blinded manner for the partial and final analysis.

The masking will be done using boxes in the identical primary packaging in the three groups. Blinding for the research subject and the researcher will be done by replacing the commercial labels in the case of the comparator in the bottles and the use of identical labels that contain the assignment number.

7.4.1 Opening of blinding.

Blinding may be opened in the following cases:

1. Presence of a serious adverse event.
2. Safety alarm due to the use of the drugs under study.
3. In case the sponsor determines it for any security reason or other reason that it considers pertinent

8. Methods Collection, administration and data analysis

8.1 Methods of data collection

A clinical monitor will be assigned to each research center, which will be authorized to monitor, review, procure and ensure that the quality of the information obtained from the participants is reliable and trustworthy. Each monitor will schedule periodic visits to the research centers in order to review the source documents and corroborate the information captured in the case report format (CRF). All clinical monitors will be trained in relation to the information of the study protocol (objective, visits, procedures, range of accepted values, etc.). In the event that the data are not identical between the two registers, the clinical monitor will generate a discrepancy, which must be resolved by the research center in time that the sponsor deems reasonable to meet the objectives of the clinical study. The correction of the discrepancies will be made according to the Good Documentation Practices.

The data registered in the CRF will be reviewed by personnel of Sophia Laboratories, trained in the ophthalmological, clinical and pharmacological area, which will be able to generate discrepancies in the event that the data do not comply with the stipulations of the research protocol or put the participants at risk.

Once all discrepancies generated by the team of clinical monitors and clinical staff have been resolved, the data will be downloaded into an electronic database (Excel Sheet) by personnel designated by the sponsor. A new review of the data will be carried out to corroborate the fidelity of the same and new discrepancies may be generated in case it was considered.

The database generated will be safeguarded by the sponsor and will only have personal access designated by the same.

8.1.1 Strategies to complete the follow-up.

- You will be clearly informed of the importance of the study and the benefits that the population will obtain from the results of the study.
- Transportation assistance will be provided in order for the participant to attend their visits.
- Calls, messages or a printed calendar will be made in order to remind the participant of their appointments and the activities that will be carried out, in addition to the estimated duration of the same.
- In case the participant does not attend his appointment, the research center must make a call to know the reason and try to arrange a new appointment within the established window period or an unscheduled appointment.
- In case it is not possible to make an appointment, it will be asked about the presence of adverse events and the reason for leaving the study, such as minimum data.

8.2 Data management.

The subject's medical record (including clinical notes, test results, etc.), as well as the subject's diary, and the ICO questionnaire are considered source data.

The IP or the designated person of your team will fill out the Case Report Format (CRF) as well as all other documents provided by the sponsor (for example, documents related to the handling of the treatment).

A CRF was designed to record the data that are required in the protocol and that the researcher collects in each of the visits.

In the case of self-assessment questionnaires, it is not permissible for the principal investigator or person responsible for filling in to modify what was written by the subject of the study.

The data capture in the investigator's site will be done by the investigator or the designated person of his team after performing the Medical File. The researcher or a designated person of your team will be trained in the filling of the CRF

All corrections to the CRF data should be made by the investigator or the designated person of your team in accordance with the instructions provided.

To ensure the confidentiality and security of the data, user names and access codes will be used to restrict access to the system only to authorized personnel.

The monitor must ensure that all the data has been filled in the CRF. After comparing the data against the source documents, the monitor will ask the researcher to make the necessary correction / clarification, so that they are answered and closed as quickly as possible.

The Scientific Committee of Sophia Laboratories, S.A. of C.V. will give the latest medical-scientific review, and will set the standard for freezing the database.

8.3 Statistical methodology

8.3.1 Analysis of primary and secondary outcome variables.

The statistical analysis will be carried out by personnel from Sophia Laboratories, S.A. of C.V The statistical program SPSS version 19 (IBM Corporation, Armonk, NY, USA) will be used.

The designated personnel will be blinded to the intervention groups. The coding will be done using consecutive numbers for each intervention group.

The data will be collected and sorted in an excel sheet. Later they will be exported to the platform of the SPSS program. The variables will be categorized according to their nature.

The result of the continuous quantitative variables will be presented in measures of central tendency: media, standard deviation and ranges. **See Table 5**. Scales to be used

The normal distribution of the results will be obtained by the Kolmogorov-Smirnov test.

The statistical analysis of the continuous **quantitative variables** to find significant differences (p) will be the following:

- Intra-group analysis: Wilcoxon rank test.
- Inter-group analysis: Kruskal-Wallis test.

The level of difference to consider significance will be an alpha of 0.05 or less.

The result of nominal and ordinal qualitative variables will be presented in frequencies, proportions and percentages. See table 1 (section 6.4.3).

The statistical analysis to identify significant differences of the **qualitative variables** will be done by creating 2x2 contingency tables and will be done as follows:

- • Intra-group difference: McNemar test.
- • Difference between groups: χ2 test (Chi-square) of Pearson.
- The level of difference to consider significance will be of an alpha of 0.05 or less.
- For the reporting of adverse events all eyes of those participants who were randomly assigned to an intervention group after the baseline visit will be considered. The results will be expressed in number of cases (eyes).
- The final report of the results will be shown in tables or graphs, as appropriate.
- It will be considered that the investigational drug is safe and tolerable when there are no clinical and statistical differences in all the variables of primary outcome, with respect to its comparators.
- Those subjects who comply with an adherence greater than 60% will be included in the statistical analysis to meet the objective of the study. It was considered that from the minimum dose necessary to obtain a pharmacological effect and the presence of adverse events (exposure) is sufficient to fulfill the general objective of the design, according to the pharmacological characteristics of the product under investigation.

8.3.2 Additional analyzes

A partial analysis will be carried out with the objective of evaluating safety and adherence once 3 subjects per group (33% of the sample size) have completed all the visits and study procedures. Non-parametric statistics will be used and the same mechanisms described for the total analysis of the data will be used.

8.3.3 Population analysis and management of missing data

An intention-to-treat analysis will be carried out, which will include the data of the participants who have completed at least two visits (Visit 1 and 2), after the baseline visit.

9. Methods Monitoring

9.1 Data monitoring

Monitoring visits by a site monitor of Sophia Laboratories, S.A. of C.V. are intended to confirm that studies sponsored by Sophia Laboratories, S.A. of C.V. they are conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practices and with the applicable regulatory requirements (verifying a continuous compliance with the protocol, amendment or amendments, reviewing accounting records of the product under investigation, verifying that the personnel of the site and the facilities remain adequate to carry out the study).

The researcher must ensure that they have sufficient time, space and qualified personnel for the monitoring visits.

In order to carry out the monitoring review, it is mandatory to provide direct access to all source data and those related to the study site. The monitor will conduct a review of the CRF and a Verification of Source Documents (VDF). By VDF means the verification of the records in the CRF through its comparison with the source data that the researcher will make available for this purpose.

Regarding the CRF, the monitor will mark in each visit the screens completed and approved in case of use of electronic platform.

In accordance with the applicable regulations, Good Clinical Practices, and the procedures of Sophia Laboratories, S.A. of C.V. The monitors of Sophia Laboratories, S.A. of C.V. they will contact the site before the start of the study to review the protocol, the regulatory and ethical requirements of Laboratorios Sophia, S.A. with the staff of the site of C.V. In reviewing the procedures for data collection, the conversation will also include the identification, agreement and documentation of the individual data for which the records in the CRF serve as source documents.

Sophia Laboratories, S.A. of C.V. will monitor the study to verify, among other things, that:

- The data is authentic, correct and complete.
- The safety and rights of the subjects are being protected.
- The study is being conducted in accordance with the currently approved protocol, any other study agreement, Good Clinical Practices and all applicable regulatory requirements.

The investigator and the head of the medical institution (when applicable) agree to allow the monitor to have direct access to all relevant documents.

Study monitoring visits will be conducted at regular intervals, depending on the recruitment rate, under the arrangements between the investigator and the sponsor. All information related to these visits will be handled as strictly confidential.

Upon completion or early termination of the study, the monitor will carry out site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations, Good Clinical Practices, and Sophia Laboratories, SA of C.V. procedures.

After the study is closed, the researcher must keep all study records on the site in a safe place. Records should be maintained to allow easy and timely recovery, when necessary (for example, in an audit or inspection). Sophia Laboratories, S.A. of C.V. will inform the investigator / institution the period of time they will have to retain these records, in order to comply with all applicable regulatory requirements. Nevertheless, the investigator / institution must seek the written approval of the sponsor before proceeding to the elimination of these records. The minimum retention time will satisfy the most stringent standard applicable to that site for the study, in accordance with the provisions of the PCBs, any institutional requirements or the applicable laws or regulations, or the standards / procedures of Sophia Laboratories, S.A. of C.V.

The researcher / institution must notify Sophia Laboratories, S.A. of C.V. Of any change in file arrangements including, without limitation, the following: file in an off-site facility, ownership transfer of records in the event the investigator leaves the site.

9.2 Preliminary analysis and early termination of the study

The partial analysis described in section 6.5 and 8.3.2 will allow the sponsor to make a decision about the early termination of the study in the event that the safety of the participants is compromised.

The early termination of the study will be considered in the following cases:

1. Presence of serious adverse events in more than 5% of the participants in each intervention group.
2. The competent authority (COFEPRIS) considers it for security alerts.
3. The Sponsor determined it for his convenience or eventualities such as: economic support, manufacturing errors, etc.
4. Lack of recruitment as expected.

In case the decision is the early termination of the clinical study, all the research centers will be informed within the first 24 hours by the available communication channels. Likewise, the corresponding authority in each country will be informed (if applicable) and the Ethics Committees involved.

Each research center has the obligation to inform the subjects that participate in the clinical study in a period no longer than 24 hours, after receiving the information from the sponsor. You must inform all the subjects involved in any phase of the study.

The result of the preliminary evaluation will be in charge of the Clinical Operations Management and the Medical Management of Sophia Laboratories, S.A. of C.V., which will have the faculty to determine the fate of the present protocol, as they deem convenient.

9.3 Adverse events

9.3.1 Investigator's responsibilities

Perform the verification of adverse events through questioning, relevant physical examination, assessment of evolution, as well as adequate medical and pharmacological management, resolution or outcome and final discharge following the definitions determined in national and international regulations. [47] [48] [49]

In case of adverse events or any event that puts the health and well-being of the patients at risk, appropriate medical attention will be provided, either at the research site or will be referred to the Hospital Center with greater resolving power with which the researcher and / or researcher site have medical care agreement. The researcher will notify the clinical monitor of the sponsor, according to the times established in the national and international regulations. In the case of serious adverse events, notify the sponsor and record the corresponding information in the case report form and in turn inform the Research Ethics Committee, the Research Committee.

The attention of the adverse events will be made according to the diagram of attention of the event (**see Figure 4. Attention of the adverse event**)

In the final report to be drafted by the Scientific Committee of the Department of Clinical Operations of Sophia Laboratories, S.A. of C.V., will include the report of adverse events in compliance with current national and international regulations. [48] [47].

9.3.1.1 Record of adverse events in the Case Report Form

The registry of adverse events considers the information concerning the identification data of the participating patient as code, age, sex, left eye, right eye.

Information about the type of adverse event, adverse reaction or suspected adverse reaction to the product under investigation or to the study medication, as appropriate. The date on which the adverse event occurs is reported, as well as in which the Investigator is aware of it, date of resolution or outcome, as applicable. The clinical diagnosis is indicated. Include in concomitant medications the therapy used for the pharmacological management of the adverse event, suspected adverse reaction, adverse reaction. Record the outcome or resolution of the event: patient recovered without sequelae, with sequelae, not recovered. Patient who presented death due to adverse reaction / adverse event, patient who presented death and it is judged that the drug could have contributed, patient who presented death and this is not related to the investigational product or drug, or indicate that it was not knows what the consequence of the event is.

Consign information about the product or drug under investigation or the drug associated with the adverse event, adverse reaction or suspected adverse reaction. As applicable, information concerning generic denomination, distinctive denomination or product code in research and / or investigational medication should be recorded, as appropriate according to the methodological design of the study, this is relevant in the case of blinded studies or those where they use placebo as comparators, since there are circumstances that justify opening the cecum to determine if the adverse event, the adverse reaction or suspected adverse reaction may be attributable to the active agent, the combination of active agents, or the substance (s). s) pharmacologically inert (s), such as vehicles or additives, as appropriate to the clinical research phase in which the development of the drug is located. It will also be necessary to record the data concerning the batch number, manufacturer laboratory, expiration date, dosage, route of administration, start and end dates of administration and / or consumption, reason for the prescription; according to whether it is a product or investigational medicine (protocol in which the patient currently participates) or is a medicine that the subject under investigation consumes for the treatment of basic concomitant diseases or used for the management of any sign or transient symptom that does not correspond to the Natural History of the pathology that motivated its entry into the research protocol.

Record the withdrawal or maintenance of the medication, investigational product or investigational medication, as appropriate. Indicate if the adverse event disappears when the investigational product or investigational medication or suspicious medication is removed (if the event is triggered). 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 that in those patients who are exposed again to the investigational product, investigational medication or medication, which had previously been suspended, if the adverse reaction or adverse event reappears.

Regarding concomitant pharmacotherapy. Indicate the generic name, the dose, the route of administration, start and end dates of its use, as well as the reason for the prescription regardless if it is consistent with the information to prescribe or technical data sheet or is used outside the regulations or of what the local, national or international regulatory entity has authorized.

Concerning the relevant clinical antecedents. The analysis of the adverse event, adverse reaction or suspicion of adverse reaction considers the information previously reported, notwithstanding the clinical context in which said harmful phenomenon occurs in the participants of the clinical research protocol, it is of special interest, so that the information about previous ailments, hypersensitivity or allergy phenomena, previous surgical procedures, laboratory analysis or cabinet exams that have been practiced on the participant, etc., that the researcher deems convenient to mention may do so. If you have enough space in the case report format, you can complement the information of your clinical note in the clinical file.

9.3.1.2 Follow up of adverse events

The IP will provide the attention and guidance of the EA that the participant presents until the end of the same, according to what is referred to the following section.

9.3.1.3 Procedures for a serious adverse event

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

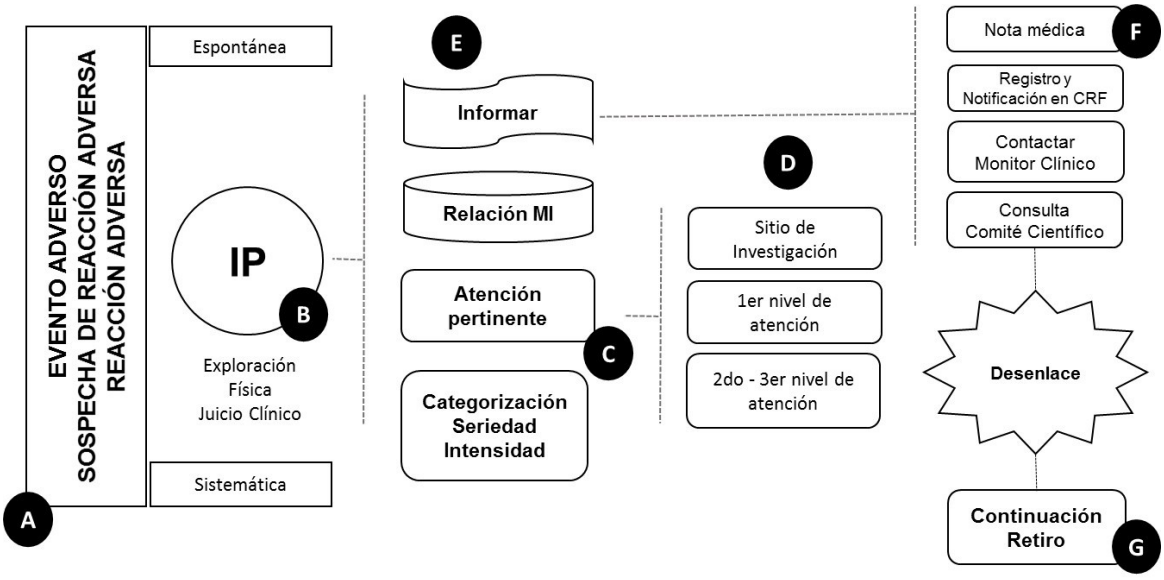


Figure 4. Attention to the adverse event

A. During the development and conduct of the present clinical investigation, undesirable damaging events or adverse reactions, of medical involvement, which do not necessarily have a causal relationship with the investigational product or investigational drug, may occur in the participant patient. These harmful phenomena can occur during the use of investigational drugs, unintentionally, at doses authorized for use in humans; by a local, national or international regulatory entity, whether for prophylaxis, diagnosis, treatment or for the modification of some physiological process. Notwithstanding, it can be suspected that the investigational product or the investigational drug or the placebo cause some unwanted clinical manifestation. Adverse events, adverse reactions or suspected adverse reactions to one or several medications 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, as such way that,

B. The investigator must be the first person to whom the patient reports that they have developed or presented a harmful clinical phenomenon during their participation in this research protocol.
C. According to their clinical judgment; on the basis of the pertinent physical examination, interrogation, etc., as well as the analysis of the information available in the medical literature and that referred to in the investigator's manual, information to prescribe or technical data sheet of the comparator drug, the principal investigator determines the relevant attention of the event / harmful reaction; either
D. in the research site or in the hospital with the greatest resolving power (1st, 2nd or 3rd level of medical attention). In such a way that, in case the patient is sent by the Investigator to a hospital, he / she attends by means of a reference system, it can be with an identification card that the patient belongs to the present investigation and there is an official number or folio, which pertains to the emergency care agreement with the health institution with the greatest resolving power, or a medical reference note issued by the principal investigator, so that appropriate care is given to the participating patient. It should be noted that the Study Sponsor, Sophia Laboratories, S.A. of C.V., will pay the expenses for the medical care of the participating patient, only if the adverse event, adverse reaction or suspected adverse reaction to medication is associated or found in relation to the investigational product or investigational drug.

E. Taking the clinical information gathered, either during the care provided in the research site or provided by the treating physician (s) in the hospital, the principal investigator records the adverse event, suspected adverse reaction or adverse reaction to medication in your clinical note of the clinical file, indicating the seriousness, intensity (mild, moderate or severe), relationship with the product or investigational medicine, as well as:
F. The migration of the relevant data to the case report format and to its respective adverse event section; noting the pertinent information, already referred to in section 9.3.1.1., this in virtue of the fact that in cases of serious adverse events, which must be notified in less than 24 hours after the moment in which the principal investigator has knowledge of the same, the clinical monitor of the study is informed, so that in turn he / she informs the Scientific Committee and the Pharmacovigilance Department of the sponsor and later he / she informs the Research Ethics Committee. Regarding non-serious adverse events, these will be recorded and adequately addressed and the corresponding regulatory entity will be informed about the safety profile of the product under investigation or investigational medication in the final report of the clinical trial.

The record of the outcome of the adverse event, suspicion of adverse reaction or adverse reaction to medication depends substantially on the follow-up that the principal investigator makes to the participant, since most of the harmful phenomena are expected, consult section of the safety profile in number 5.3 and in the researcher's manual, they are ophthalmic in nature, nevertheless there may be systemic alterations. Therefore, in the opinion of the researcher, the withdrawal of the participant or his / her permanence will be considered, according to the stipulations of section 6.2.2 Exclusion criteria of the present research protocol.

9.3.1.4 Causality evaluation

The assessment of the causality, the methodology used to estimate the probability of attributing to a drug, investigational drug or investigational product the adverse reaction, the suspicion of the same or the observed adverse event, considers probabilistic categories, according to the evidence available and the quality of information, based on national pharmacovigilance regulations. [47] As a tool to facilitate the probabilistic categorization of causality, the principal investigator can use the algorithm of Karch and Lasagna modified by Naranjo referred by Aramendi I, 2011 in which different items are qualified which allow assigning a value to the relationship cause-effect between the administration of the drug and the adverse reaction. [50] **See Table 7. Algorithm of Karch and Lasagna modified by Naranjo**

Algorithm of Karch and Lasagna modified by Naranjo

No.	Reagent	Score	
		Yes	No
1.	There are previous conclusive reports about the adverse drug reaction, adverse event or suspected adverse drug reaction	+1	0
2.	The adverse event appeared when the suspected drug was administered	+2	-1
3.	Adverse reaction to medication, adverse event or suspected adverse drug reaction improved upon discontinuation or administration of a specific antagonist	+1	0
4.	Adverse reaction to medication / adverse event / suspected adverse drug reaction reappeared when administering the drug / investigational product / investigational medication	+2	-1
5.	There are alternative causes that may cause this reaction	-1	+2
6.	Adverse reaction / adverse event / suspected adverse drug reaction occurred after placebo administration	-1	+1
7.	The drug was determined in blood or other liquids in toxic concentrations	+1	0
8.	The intensity of the adverse reaction / adverse event / suspected adverse drug reaction was higher with higher doses or lower with lower doses	+1	0
9.	The patient has had similar reactions with the drug / product under investigation or investigational medication, in the past	+1	0

Algorithm of Karch and Lasagna modified by Naranjo			
No.	**Reagent**	**Score**	
		Yes	**No**
10.	Adverse reaction / adverse event / suspected adverse reaction to medication was confirmed with some objective evidence	+1	0
	Total score	summation	
Probabilistic category based on the score obtained			
I	The causal relationship is checked	≥,9	
II	It is likely that ADR is due to the drug or product under investigation	5 a 8	
III	It is possible that the RAM is due to the drug or product under investigation	1 a 4	
IV	The causal relationship is doubtful	0	

The reagents considered by the algorithm of Karch and Lasagna modified by Naranjo where each one receives a defined score are shown and the final summation allows estimating the probabilistic category of the cause-effect relationship between the administration of the drug / product in research / investigational medicine and the adverse reaction, adverse event or suspected adverse reaction. Consider that if the information is not available, a score equal to zero is recorded.

Table 7. Algorithm of Karch and Lasagna modified by Naranjo.

In such a way that the degree of certainty to establish the investigational product or investigational medication (as appropriate) as the causal agent of the harmful phenomenon that befalls the participating patient, can be directly indicated by the principal investigator based on his or her clinical experience or well through the voluntary application of the tool mentioned previously. Nevertheless, it is important that the investigator take into account the following arguments in favor of the causal relationship:

b. Force of association that refers to the number of cases in relation to those exposed.
c. The consistency of the data, ie the presence of a common characteristic or pattern.
d. The exposure-effect pattern: which determines the relationship with the site of onset, time, dose and reversibility after suppression.
e. The biological plausibility: which refers to the possible pharmacological or physiopathological mechanisms involved in the development or presentation of the adverse event.
f. Experimental findings: for example the appearance of anomalous metabolites or high levels of drug or the product of its biotransformation.
g. Analogy: experience acquired with other related drugs, adverse reactions frequently produced by the same family of pharmacological agents.
h. Nature and characteristics of the data: objectivity, accuracy and validity of the relevant documentation. [51]

9.3.2 Responsibilities of the sponsor.

The sponsor will be responsible, and will cover the expenses derived from the medical attention to adverse events related to the product under investigation.

9.4 Audit

To guarantee compliance with the PCBs and with all applicable regulatory requirements, Sophia Laboratories, S.A. of C.V. could carry out a quality assurance audit. Regulatory agencies could also carry out a regulatory inspection of this study.

9.4.1 Pre-study audit

The research centers 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.

9.4.2 Audit / Inspection during the conduction of the study

They may take place at any time before, during or after the conclusion of the study. If an audit or inspection is performed, the investigator and the institution should agree to allow the auditor / inspector direct access to all relevant documents, and will allocate their time and that of their staff to the auditor / inspector to discuss the findings and any relevant problems.

10. Ethical considerations

10.1 Approval of the committees

The present study will be conducted according to the standards of the Declaration of Helsinki, World Medical Association 2013. Nuremberg Code; Nuremberg Trial by the International Court of Nuremberg, 1947. Belmont Report, National Commission for the Protection of Subjects of Biomedical Research and Conduct, 1979. Will be conducted in accordance with the scientific and technical requirements necessary for the registration of medicines for use of the International Conference on Harmonization (The International Council for Harmonization, ICH) Guide to Good Clinical Practices. International Ethical Guidelines for Biomedical Research in Human Beings of the Council for International Organizations of Medical Sciences (Council for International Organizations of Medical Sciences, CIOMS, 2002). International Ethical Guidelines for Epidemiological Studies of the Council for International Organizations of Medical Sciences (Council for International Organizations of Medical Sciences, CIOMS, 2008). The Research Ethics Committee and the Research Committee will evaluate the protocol before conducting the study and will issue their approval or possible modifications for its realization, these Committees should be notified of any significant changes to the protocol. In addition to the above, the current regulations issued by the Ministry of Health will also be complied with. General Health Law, NOM 012 Official Mexican Standard NOM-012-SSA3-2012, Which establishes the criteria for the execution of research projects for human health. The study is considered as an investigation with a risk greater than the minimum according to the Regulation of the General Health Law on Health Research, Title Two, Chapter I, Article 17, Category III, published in the Official Gazette on 6 January 1987.

The principal investigators or study coordinators or personnel authorized by the sponsor will be evaluated by the Research Ethics Committees, Research Committees, and when applying to the Biosafety Committee the essential documentation of the research project: research protocol, letter

of informed consent, researcher's manual, subject's diary, as well as those requested, in addition, according to local, national or international requirements applicable by regulatory entities.

The study will not start in the research site if you do not have the confidentiality agreements and economic proposal of each of the principal investigators, duly signed and without having previously obtained the favorable opinion and / or the approval of the Committees of Ethics in Research, Research Committees, and when applicable by the Biosecurity Committee, corresponding.

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

10.2 Amendments to the protocol

The amendment procedure will be relevant 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 when identifying risks in the research subjects . The documents susceptible of amendment will be: protocol, letter of informed consent, researcher's manual, documents for the patient, scales of measurement 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 Committee of Inquiry. Biosafety, (entities that issued the initial favorable opinion for the conduct of the investigation) will be sent (s) for authorization by the relevant regulatory entity.

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

The principal investigator has the responsibility to inform the Research Ethics Committee of any amendment to the protocol that could eventually affect the rights, safety or welfare of the research participants. Likewise, he must know any situation or new knowledge that shows a greater risk for the participants, the termination or premature suspension of the study, the reasons and the results obtained up to that moment. You must also inform about the conclusion of the study, when completing the research protocol.

The list of amendments, and in the necessary cases, the relation of the issuance of errata, will be referred to in the final report of the investigation.

10.3 Consent (assent))

10.3.1 Obtaining

Informed consent must be obtained before the subject undergoes any procedure indicated in the protocol.

The written consent documents will incorporate the elements of informed consent described in the Declaration of Helsinki and the ICH Guide to Good Clinical Practices and will be in compliance with all applicable laws and regulations.

The IP will provide the potential participant with all the information regarding the characteristics of the study, its potential benefits, risks, objectives and procedures thereof.

This information will be with a language understandable to the subject, it will be explained to the subject that has the right to interrupt their participation in the study at any stage, without affecting the relationship with the researcher and / or their future assistance. The informed consent will be put to the consideration of the possible participant; this must have enough time to analyze each and every one of the aspects mentioned above and if there is any doubt this 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 informed consent letter in the presence of two witnesses who have or are not related to the subject of study, who will participate during the informed consent process and will sign endorse that the process was carried out prior to any study procedure, that the information of the study was clearly explained and doubts were clarified in case of existing.
If a subject is illiterate, the acceptance will be with their fingerprint, and in the event that the subject is not able to grant an informed written consent, a representative of the "legally authorized" subject can provide such consent. The subject in accordance with applicable laws and regulations.
The IP must also sign and date this consent.
The informed consent must be signed in duplicate by all involved, and two witnesses, one copy will be filed in the file of the subject and the other will be delivered to the participant. The PI must document in the patient's medical history, the date on which he signed the informed consent.

10.3.2 Special considerations

The auxiliary studies that will be carried out during the conduction of the study (laboratory tests) do not pose an additional risk that should be considered apart from the procedures listed in the informed consent.

10.3.3 Modification to informed consent

Any change to "informed consent" constitutes an amendment to this document and must be submitted for approval to the Research Ethics Committees, and if applicable before the Competent Authorities.

The amendment will include a copy of the new version in the language or languages of the country.

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

Each subject affected by the amendment must complete, date and sign two originals of the new version. The subject will be given a signed original of the amendment and the researcher will keep the second original.

10.4 Confidentiality

All documents and information provided to the researcher by the sponsor are strictly confidential. The researcher expressly agrees that the data on their professional and clinical experience, provided to the sponsor on paper and stored in electronic format, are only for use related to their activities with the sponsor of clinical studies, in accordance with Good Clinical Practices. The researcher accepts that he / she and the members of his team will use the information only within the

framework of this study, to carry out the protocol. This agreement is mandatory as long as the confidential information has not been disclosed to the public by the sponsor. The protocol of the clinical study provided to the researcher may be used by him and by his colleagues to obtain the informed consent of the subjects for the study. The clinical trial protocol, like any information taken from it, should not be disclosed to other parties without the written authorization of the sponsor.

The researcher will not reveal any information without the prior written consent of Sophia Laboratories, S.A. of C.V., except to the representatives of the Competent Authorities, and only by request of the same. In the latter case, the researcher undertakes to inform Sophia Laboratories, S.A. of C.V. before revealing the information to these authorities.

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

10.5 Declaration of interests

The PI is committed to making a declaration of financial interests, as well as a conflict of interests prior to the start of the study..

10.6 Access to information

The final database of the study will be owned by Sophia Laboratories, S.A. of C.V. and your access will be restricted. The IP will not have access to it, unless it has prior written authorization from the sponsor.

10.7 Auxiliary care and after the end of the study

Once the study is completed and the adverse events are closed according to section 9.3 Adverse events, the sponsor will not extend care on the research subject.

10.8 Biosecurity aspects

WITHOUT BIOSECURITY IMPLICATIONS

The present protocol, with the title: "Phase I clinical study, to evaluate the safety and tolerability of the preservative-free ophthalmic solution PRO-087 versus Xyel Ofteno® and Systane Ultra®, on the ocular surface of ophthalmological and clinically healthy subjects", and number: SOPH087-0616 / I DOES NOT HAVE BIOSECURITY IMPLICATIONS, since infectious-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 personnel of the research center or the subjects of investigation or affects the environment. In addition, it is stated that cell, tissue or organ transplant procedures or cell therapy procedures will not be carried out in this project, nor will laboratory, farm or wildlife animals be used.

10.9 Final report and publication of results

10.9.1 Final report

Once the statistical analysis is finished, a final report will be drafted with the results obtained, in charge of the Scientific Committee of the Department of Clinical Operations of Sophia Laboratories,

S.A. of C.V. Said report will be prepared following the recommendations of the E3 Step 4 Guide of the ICH.

10.9.2 Communication of results

Regardless of the results in the study, Laboratorios Sophia, S.A. de C.V., is committed to communicate the final report of the study to the principal investigators and to the corresponding regulatory entities of the countries with participating research centers. Maintaining at all times the rights on the publication and dissemination of the information contained.

10.9.3 Publication of the results

Sophia Laboratories, S.A. 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 may be used in the manner it deems appropriate.

The PI undertakes not to publish or communicate data collected only in a center or in part of the centers before the publication of the full results of the study, unless prior written agreement is given by Sophia Laboratories, S.A. of C.V.

Any publication and / or communication project related to the study and / or the results obtained during the study or after the completion of the study will be presented to participating medical researchers at least 30 days in the case of a publication and 15 days in the case of a summary, before the scheduled date for the communication and / or presentation of a publication. The medical researcher or doctors will comment on the project within 15 days in the case of a publication and 7 days in the case of a summary, from the date on which the project is received.

Nevertheless, in case the sponsor is in the process of submitting 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.

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12. Signature page

Medical manager of the study:

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Internal reviewer of the protocol:

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External reviewer of the protocol:

Dr. Mariana Xacil Díaz Salazar

Principal investigator of the study:

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13. Annexes

13.1 Eye comfort index

Ocular Comfort Index

ID:

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						Always
0	1	2	(3)	4	5	6

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						Always
0	1	2	3	4	5	6

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

Never had it						Severe
0	1	2	3	4	5	6

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

Never						Always
0	1	2	3	4	5	6

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

Never had it						Severe
0	1	2	3	4	5	6

3 In the last week, how often did your eyes feel *stingy* ?

Never						Always
0	1	2	3	4	5	6

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

Never had it						Severe
0	1	2	3	4	5	6

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

Never						Always
0	1	2	3	4	5	6

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

Never had it						Severe
0	1	2	3	4	5	6

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

Never						Always
0	1	2	3	4	5	6

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

Never had it						Severe
0	1	2	3	4	5	6

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

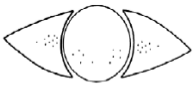
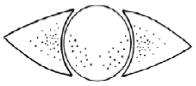
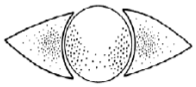
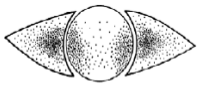
Never						Always
0	1	2	3	4	5	6

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

Never had it						Severe
0	1	2	3	4	5	6

13.2 Efron scale for conjunctival hyperemia

13.3 Oxford Scale

PANEL	Grade	Criteria
A 	0	Equal or less than panel A
B 	I	Equal to or less than panel B, greater than A
C 	II	Equal to or less than panel C, greater than B
D 	III	Equal to or less than panel D, greater than C
E 	IV	Equal to or less than panel E, greater than D
>E	V	Greater than panel E