Excelencia en oftálmicos

Study protocol:

SOPH037-1219/II

Title: Phase II clinical study to evaluate the efficacy of the multi-dose ophthalmic solution Lagricel® Ofteno in 3 different dosages for the management of mild to moderate dry eye

Information about the molecule under study

Generic name: Sodium hyaluronate 0.4%

Distinctive name Multi-dose Lagricel® Ofteno

Indication: Ocular lubricant

Information of the protocol

Study phase: II

Version: 2.0

Release Date: Feb 20, 2020

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

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

Changelog

Changes from version 1.0, date 04-Dec-19 to version 2.0, date 20-Feb.20

1. Page 18, Schedule of activities. Fill training is added in the section corresponding to subject's diary delivery.
2. Page 31, Specific Objective. The wording is modified. It is located within the section of Secondary objectives, it is eliminated in this last section.
3. Page 31, Secondary Objectives. First secondary objective is removed, it is located within Primary Objective. Wording is modified to combine the 3 dosages in each objective.
4. Page 43, Section 6.3. The restriction of the use of unauthorized drugs during the intervention period is added the specification of: Including the period between the end of the Basal Visit (BV) and Day 1 (D1), from D1 the scheme of applications of the Investigational Product (IP) will begin. The following information is added: The use of hormonal contraceptives is authorized; At the discretion of the investigator, systemic medicines whose effects do not modify the efficacy or safety parameters of this protocol may be used, but they must be notified to the sponsor's scientific committee to determine the participant's status as appropriate. Prohibited medicines section is added: Any ophthalmic medicine that is not on the list of allowed medicines. Systemic medications: steroids, immunomodulators and tetracyclines.

Contents

Study leaders

The administrative structure of the sponsoring party, corresponding to Laboratorios Sophia, S.A. de C.V., is shown in Table 1. Study leaders.

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

¥ Employees of Laboratorios Sophia, S.A. de C.V., Av. Paseo del Norte No. 5255, Col. Guadalajara Technology Park, Guadalajara-Nogales Highway Km 13.5 CP 45010 Zapopan, Jalisco, Mexico Tel +52(33) 3000 4200

Table 1. Study leaders

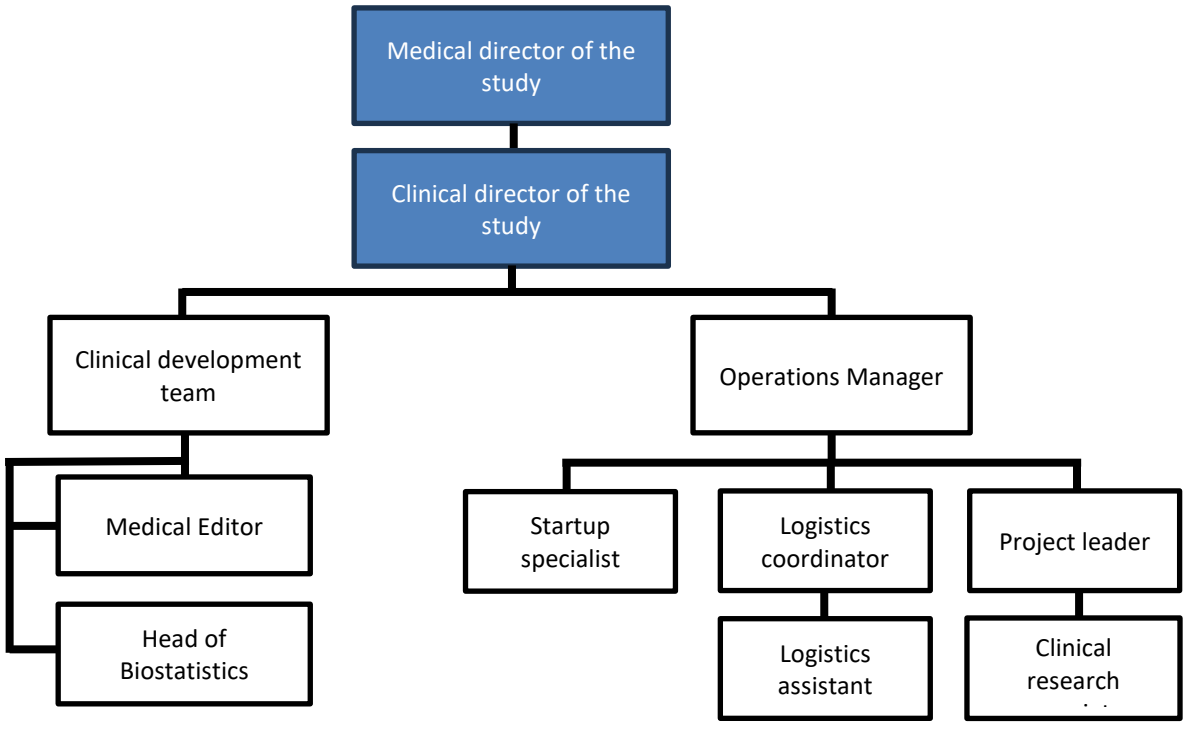


Figure 1. Administrative structure

Researcher Agreement

I agree to conduct this clinical study in accordance with the design and guidelines of this protocol, in accordance with the provisions of this protocol and in accordance with the accepted standards of Good Clinical Practice.

I agree to report all information or data in accordance with what is indicated in the protocol, in particular, any adverse events. I also agree to handle clinical supplies, provided by the sponsor, strictly in accordance with this protocol.

I understand that the information that identifies me may be used by the sponsor. Because the information contained in this protocol and the Investigator's Manual is confidential, I understand that it is prohibited from sharing it with any third party, which is not involved in the approval, supervision, or conduct of the study. I will ensure that I take the necessary precautions to protect the information from loss, inadvertent disclosure, or access by unauthorized third parties.

Name:

[Write the full name of the researcher]

Qualification:

Principal Investigator

Name of the center:

[Write name of study center]

Geographic location (city/state/country)

[Write the geographic data of the center]

Signature

Date

List of abbreviations

BID	Twice a day (As per its abbreviation in Latin, *bis in die*).
CONSORT	Consolidated Standards of Reporting Trials
UFTLS	Pharmacovigilance and Technovigilance Unit of Laboratorios Sophia, S.A. de C.V. (As per its abbreviation in Spanish, Unidad de Farmacovigilancia y Tecnovigilancia de Laboratorios Sophia S.A. de C.V.)
REC	Research Ethics Committee
RC	Research Committee
COFEPRIS	Federal Commission for the Protection against Sanitary Risks (As per its abbreviation in Spanish, *Comisión Federal para la Protección de Riesgos Sanitarios*).
D	Day
IUD	Intrauterine device
AE	Adverse Event
eCRF	electronic Case Report Form
OSD	Ocular surface disease
ICF	Informed consent form
FDA	Food and Drug Administration of the United States of America
SH	Sodium Hyaluronate
CI	Confidence interval
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
PI	Principal Investigator
ITT	*Intention To Treat* population
SC	Safety call
BCVA	Better corrected visual acuity
mmHg	Millimeters of mercury
n	Number
OU	Both eyes (As per its abbreviation in Latin, *oculus uterque*)
WHO	World Health Organization
OSDI	Ocular Surface Disease Index

IOP	Intraocular pressure
IP	Investigational Product
UAPs	Unanticipated problems
PP	*Per protocol* population
PRN	*Pro re nata*
QID	Four times a day (As per its abbreviation in Latin, *quater in die*).
RNEC	National Clinical Trials Registry (As per its abbreviation in Spanish, *Registro Nacional de Ensayos Clínicos*)
6x/D	Six times a day
SICCA	Sjögren International Clinical Collaboration Alliance
ET	Evaluation time
TID	Three times a day (As per its abbreviation in Latin, *ter in die*).
TFOS DEWS I	Workshop on dry eye of tear film and ocular surface societies I
TFOS DEWS II	Workshop on dry eye of tear film and ocular surface societies II
TFRT	Tear film rupture time
NI-TFRT	Non-invasive tear film rupture time
BV	Basal visit
V1	1st follow-up visit
FV	Final visit

1. Summary

5.1 Synopsis

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

Phase II clinical study to evaluate the efficacy of the Multi-dose Lagricel® Ofteno ophthalmic solution in 3 different dosages for the management of mild to moderate dry eye</td></tr>
<tr><td>Study Number:

SOPH037-1219/II</td><td>Date of creation:

Dec 4-19</td></tr>
<tr><td>Protocol version:

2.0</td><td>Release Date:

February 20, 2020</td></tr>
<tr><td>Therapeutic indication:

Eye lubricant</td><td>Use:

Dry eye</td></tr>
<tr><td>Estimated duration of the study (from the first patient's first visit to the preparation of the final report):
7 months</td><td>Clinical Development Phase: II</td></tr>
<tr><td colspan="2">Objectives:

Main objective:

• To assess the efficacy of 3 dosages of Multi-dose Lagricel® Ofteno in patients with mild to moderate dry eye by improving OSDI score.

Specific objective:

• To assess the safety of 3 doses of Multi-dose Lagricel® Ofteno in patients with mild to moderate dry eye by means of the incidence of unexpected adverse events (AEs) related to the investigational product.</td></tr>
<tr><td colspan="2">Hypothesis:

H_0: Multi-dose Lagricel® Ofteno decreases the OSDI test score (Ocular Surface Disease Index) by 20% or more at the end of the study in at least 1 of the proposed dosages.

$H_0: P_A - P_B \leq \delta$

H_1: Multi-dose Lagricel® Ofteno does not decrease the OSDI test score by 20% at the end of the study in at least 1 of the proposed dosages.

$H_1: P_A - P_B > \delta$</td></tr>
</table>

Study Design:

Phase II, multicenter, comparative, controlled, parallel-group, open-label, randomized clinical trial.

Number of subjects (planned and analyzed):

n = 141 evaluable patients.

47 evaluable patients per group (3 groups in total) (both eyes [OU]).

Diagnosis and main inclusion criteria:

- Patients with mild to moderate dry eye.

Selection criteria:

Inclusion criteria:

- Be of legal age.
- Have the ability to voluntarily give their signed informed consent.
- Be able and willing to comply with scheduled visits, treatment plan, and other study procedures.
- Be willing to modify lifestyle activities.
- Women of childbearing potential must ensure continued use (initiated ≥ 30 days prior to signing the Informed Consent Form [ICF]) of use of a hormonal contraceptive method or intrauterine device (IUD) during the study period.
- Have a diagnosis of mild to moderate dry eye, defined by:
  - OSDI from 13 to 32 points

or + 1 of the following:

- Corneal staining with more than 5 sites.
- Conjunctival staining with more than 9 sites.
- Tear film rupture time (TFRT) < 10 seconds.

Exclusion criteria:

- Be pregnant, breastfeeding, or planning to become pregnant within the study period.
- Have participated in another clinical research study ≤ 30 days prior to the scrutiny.
- Have previously participated in this study.
- Have a better corrected visual acuity (BCV) of 20/200 or worse in one of the eyes.
- Present an ophthalmological diagnosis of:
  - Allergic, viral, or bacterial conjunctivitis.
  - Anterior blepharitis.
  - Parasitic eye infections (e.g., Demodex).
  - Unresolved eye trauma.

- Healing diseases of the ocular surface.
- Corneal or conjunctival ulcers.
- Filamentous keratitis.
- Neurotrophic keratitis.
- Bullous keratopathy.
- Neoplastic diseases on the ocular surface or adnexa.
- Diseases with fibrovascular proliferations on the conjunctival and/or corneal surface.
- Retinal and/or posterior segment diseases that require treatment or threaten visual prognosis.
- Glaucoma.

- Have a management of dry eye that requires the implementation of stage 2 treatments of the TFO DEWS II (Tear Film and Ocular Surface Societies II Dry Eye Workshop) Recommendations on Treatment and Staged Management for Dry Eye Disease.
- Have a current history of drug addiction or drug dependence, or within the last two years prior to signing the ICF.
- Have a history of eye surgical procedure within the last 3 months prior to signing the ICF.
- Be a soft or hard contact lens wearer. You will be able to enter if you can suspend its use during the study, and must complete 15 days without using the contact lens prior to your inclusion.
- Have another medical condition, acute or chronic, that in the PI's judgment may increase the risk associated with participation in the study or administration of the investigational product, or that may interfere with the interpretation of the results of the study.
- Have known hypersensitivity to the components of the products in research.

Investigational Product (IP):

- Multi-dose Lagricel® Ofteno. Sodium hyaluronate 0.4%. Ophthalmic solution. Laboratorios Sophia, S.A. de C.V.
- Dosage per group:
  1. Group 1: 1 drop 2 times a day (BID), OU.
  2. Group 2: 1 drop 4 times a day (QID), OU.
  3. Group 3: 1 drop 6 times a day (6x/D), OU.
- Route of administration: Topical ophthalmic

Duration of treatment:	**Duration of the subject in the study:**
30 days	Up to 34 days

Evaluation criteria:

Primary outcome variable of efficacy:

- OSDI test score (Evaluation Time [ET]: day 31).

Secondary outcome variables of efficacy:

- Changes in the TFRT (ET: day 15 and 31).
- Changes in corneal and conjunctival staining with lissamine green (ET: day 15 and 31).
- Corneal and conjunctival staining changes with fluorescein (ET: days 15 and 31).
- Conjunctival hyperemia changes (ET: day 15 and 31).
- Incidence of chemosis (ET: days 15 and 31).

Safety primary outcome variable:

- Incidence of AEs (ET: days 15 and 31).

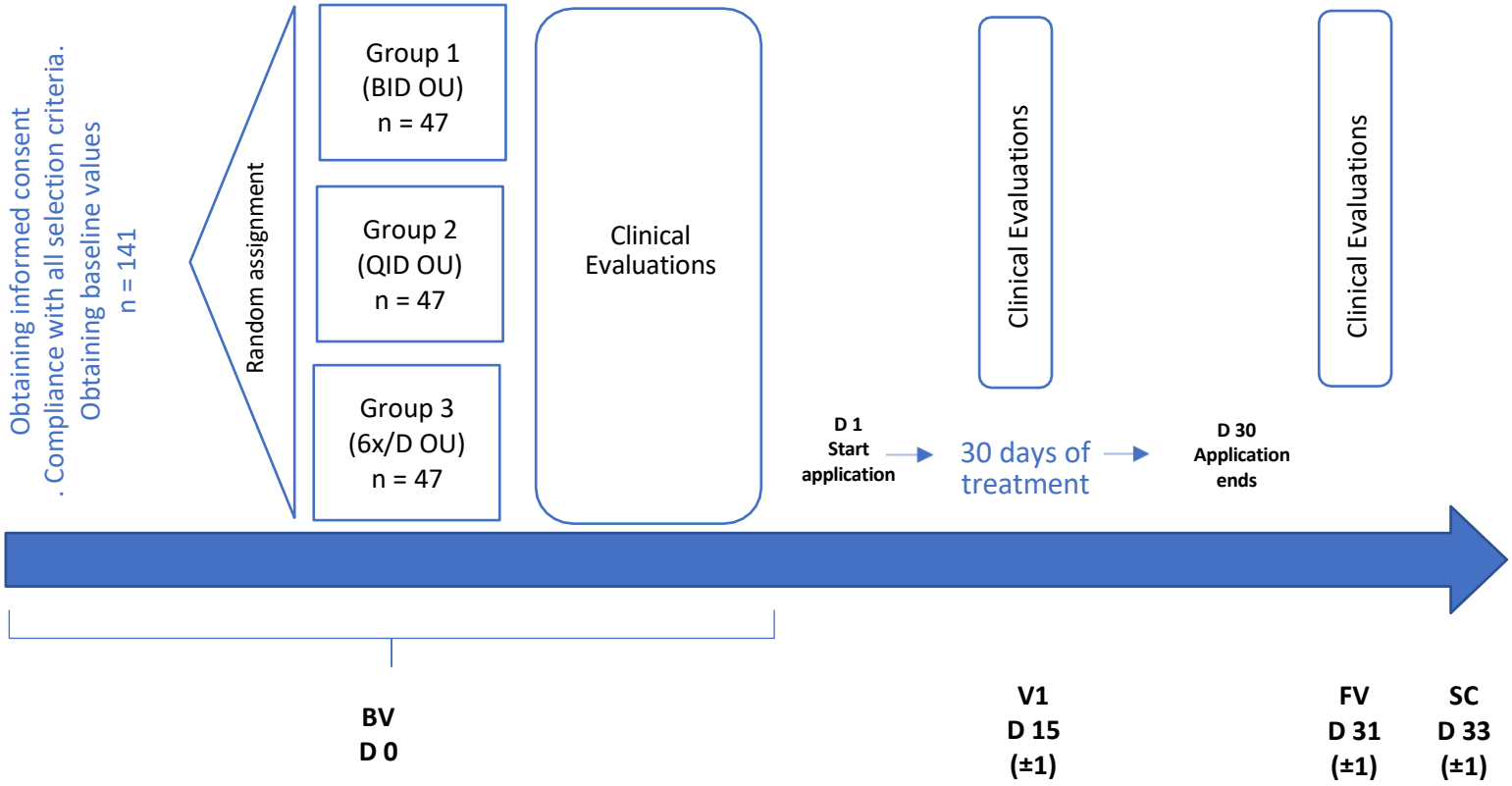
Safety Secondary Outcome Variable:

- Changes in the BCVA (ET: day 15 and 31).
- Changes in intraocular pressure (IOP) (ET: day 15 and 31).

Statistical methodology

The Kolmogorov-Smirnov and Shapiro Wilk tests will be performed, as appropriate, to know if the distribution presents normality in the results obtained in each study group. The data will be expressed with measures of central tendency: mean and standard deviation for the quantitative variables. Qualitative variables will be presented in frequencies and percentages. The statistical analysis will be performed by means of the Analysis of Variance Test (ANOVA) for the quantitative variables for the difference between the groups. The difference between the qualitative variables will be analyzed by means of X2 (Chi-square) or Fisher's exact. A 95% CI will be considered for the non-inferiority criteria based on the objectives of the study, an alpha (α) will be considered significant≤ 0.05

1.2 Study Diagram



BV: basal visit, **V1:** 1st follow-up visit, **FV:** final visit, **SC:** safety call.
BID: Twice a day, **QID:** four times a day, **6x/D:** six times a day.
OU: both eyes, **D:** day, **n:** number.
***** The parentheses () indicate the allowed window period.

Figure 2. Diagram of the study.

1.3 Study Schedule

PROCEDURES	VB D 0	D1	V1 D 15 (±1)	VF D 31 (+1)	SC D 33 (+1)
ICF Signature	X				
Medical history	X				
Drug Evaluation Concomitant	X		X	X	
Urine pregnancy test	X			X	
Vitals	X		X	X	
BCVA	X		X	X	
TFRT Evaluation	X		X	X	
Ocular surface integrity (Stains and evaluation of hyperemia and conjunctival chemosis)	X		X	X	
Comprehensive ophthalmological evaluation	X		X	X	
IOP	X		X	X	
Eligibility Criteria	X				
AE Assessment	X		X	X	X
Assignment of Investigational Product (IP)	X				
OSDI test	X			X	
Delivery of the IP	X				
Start of intervention	X				
Delivery of subject's diary and filling instructions	X				
Adherence assessment			X	X	
Return / Evaluation of the subject's diary	X				
Return of the IP	X				

***** The parentheses () indicate the allowed window period.*

Table 2. Study schedule.

2. Introduction and Background

5.2 Framework

Dry eye is defined as a multifactorial disease of the ocular surface, characterized by loss of homeostasis in the tear film accompanied by ocular symptoms, in which instability, hyperosmolarity, inflammation and damage of the ocular surface, as well as neurosensory abnormalities play etiological roles. [1]

Dry eye is a common disease, whose estimated prevalence varies widely depending on its classification [2], for example the initial report of the epidemiology subcommittee of the TFOS DEWS I concluded that the global prevalence of dry eye in individuals over 50 years of age was between 5-30%. [3]. While the report of the epidemiology subcommittee of the TFOS DEWS II marked a prevalence of 5-50% in studies involving symptoms, with or without signs; For studies in which the diagnosis was mainly based on signs, the prevalence was even higher, reaching up to 75%. [4]

The incidence of the disease has been reported in few studies. The *Beaver Dam Eye Study* established, in the Caucasian population aged 48-91 years, that 13.3% (95% CI 12.0 – 14.7%) of individuals develop symptomatic dry eye at 5 years and 21.6% (95% CI 19.9 – 23.3%) at 10 years. Age was a risk factor for the increase in incidence with an odds ratio of 1.2x (1.1 – 1.3) for each increase of 10 years. [5]

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 dry eye in the Hispanic population when compared to the Caucasian population. [6] [7]

The tear film, composed of various substances such as lipids, proteins, mucins, and electrolytes, [8] plays an essential role in lubricating and protecting the ocular surface, as well as maintaining a smooth refractive surface for optimal visual performance. [9]

When applying the concept of homeostasis to dry eye, the concept recognizes the possibility of the numerous changes that can occur in the tear film and ocular surface in response to one or more underlying causes of dry eye. [9, 10]

Figure 3 incorporates a clinical decision algorithm based on current knowledge of the pathophysiology of dry eye.

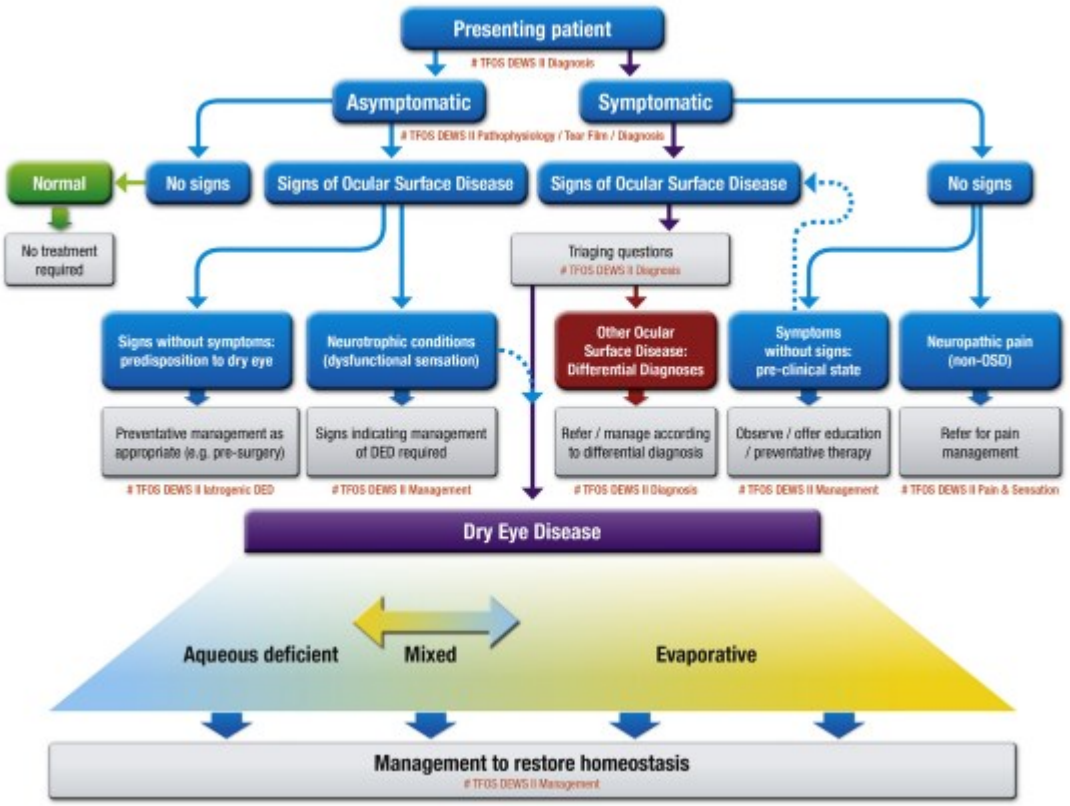


Figure 3. Classification of dry eye

Taken from the TFOS DEWS II Definition and Classification Report [1]

Artificial tears are traditionally the most commonly used therapeutic agent in dry eye. [8] Tear substitutes are composed of a wide variety of compounds that improve the viscosity of the tear film and seek to restore the natural tear film, usually aiming to act on one or more of the layers of the tear film. [8] However, these products do not act on the underlying pathophysiology of dry eye, they only partially attenuate ocular symptomatology, unfortunately the mechanisms of any palliative action are generally poorly understood. [11]

The wide variety of properties of ocular lubricants has been described in other works. [12, 13, 14, 15, 16]

Taking into account that the loss of tear film homeostasis and inflammation of the ocular surface are part of the dry eye process, together with the need for long-term management, the choice of the topical ocular formulation indicated as treatment should try to avoid, as far as possible, unwanted side effects that *per se* alter the tear film or induce an ocular inflammatory response [2].

Eye lubricants are largely considered safe, although there are some reported side effects, mainly blurred vision, varying levels of "eye discomfort," and foreign body sensation [17].

There are relatively few randomised controlled trials that have compared the relative superiority of a given product against other products for the treatment of dry eye. [18]

A recent Cochrane systematic review, which aimed to assess the effect of eye lubricants for the treatment of dry eye, included 43 randomised controlled trials that had compared various lubricant formulations with no treatment or with a placebo [17]. The primary outcome measure was patient-reported symptoms. The authors reported that the overall quality of the evidence was low for the different teardrop supplement formulations compared in the review, and concluded that although artificial tears may be effective for the treatment of dry eye, there was still a need for future research to allow robust conclusions to be drawn about the effectiveness of ophthalmic lubricant formulations.

The management of dry eye is complicated due to its multifactorial etiology. Developing the simple principle that "diagnosis precedes treatment" means that doctors should do their best to identify the degree to which the Ocular Surface Disease (OSD) contributes to the presentation of symptoms. This aspect of determining the main causative factors of dry eye is critical for proper management.

Figure 4 presents, schematically, an approach to the management of dry eye. Before selecting the appropriate treatment, a directed interrogation (Table 1) should be performed and diagnostic tests should be performed in order to determine whether the patient actually has dry eye and whether he or she is generally showing more signs of OSD. After the diagnosis is confirmed, the severity of the disease, together with the determination of the etiological subtype, an appropriate treatment plan can be developed (Table 2).

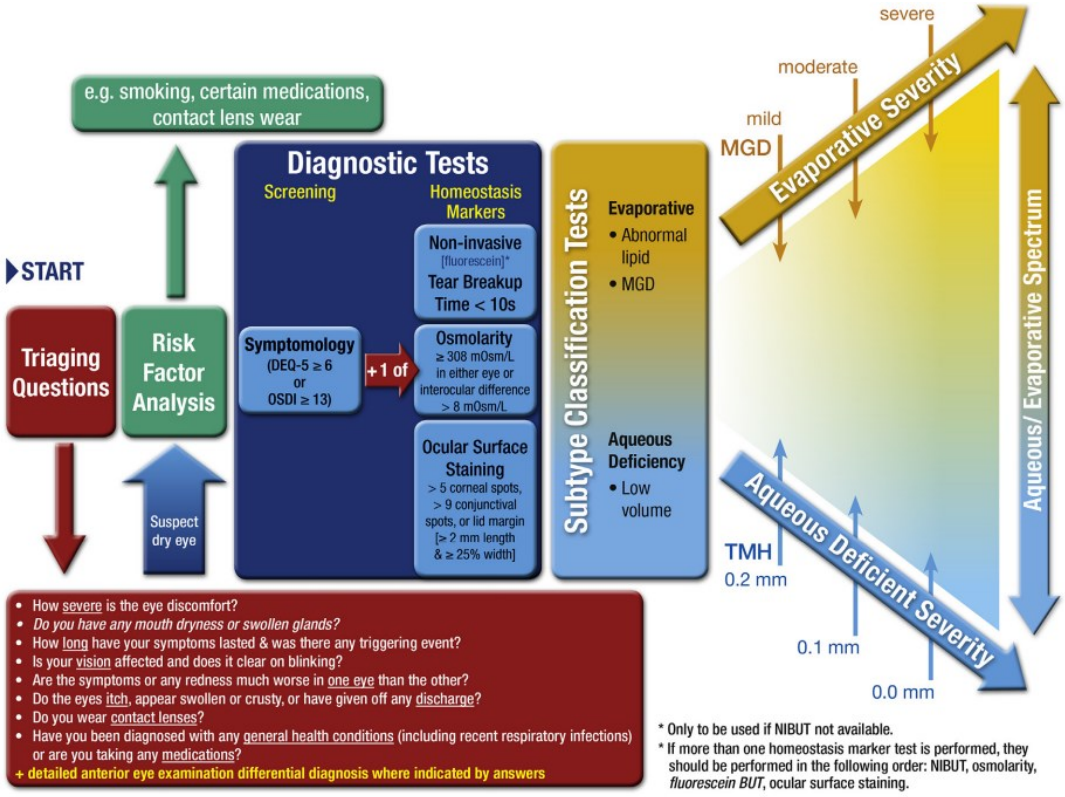


Figure 4. Diagnosis and management of dry eye

*Fluorescein is used when NI-TFRT is not available. * If more than one homeostasis marker test is performed, the order should be: NI-TFRT, osmolarity, TFRT with fluorescein, and stains. Taken from the TFOS DEWS II Management and Treatment Report [1]

- How severe is eye discomfort?
  - Do you have a dry mouth or have you had swollen glands?
- ¿How much time have Lasted their symptoms Was some trigger?
- Has your vision been affected, does it clear when you blink?
- Are the symptoms or redness in one eye much worse than in the other?
- Do your eyes itch, appear swollen, crusty, or discharge?
- Do you wear contact lenses?
- Have you recently been diagnosed with any general illnesses, or do you take any medications?

+ Detailed eye examination

Table 1. Triage Questions

Stage 1:

- Education according to the disease, its management, treatment and prognosis
- Modification of the local environment
- Education according to dietary modifications
- Identification and modification/elimination of topical and systemic offending medications
- Eye lubricants
- Eyelid hygiene and warm compresses

Stage 2:

If the above options are inadequate, consider the following:

- Treatment for Demodex
- Tear Conservation
    - Lacrimal Occlusion
    - Wet Camera Goggles
- Nighttime treatments (e.g. ointments)
- Physical warm-up and expression of meibomian glands
- Pulsed Light Therapy
    - Antibiotics or their combination with steroids on palpebral margins
    - Topical steroids (limited duration)
    - Topical immunomodulators
    - Topical LFA-1 antagonists
    - Topical macrolides or tetracyclines

Stage 3:

- Oral secretagogues
- Autologous serum
- Therapeutic contact lenses

Stage 4:

- Topical steroids (extended duration)
- Amniotic membrane grafts
- Surgical occlusion of puncta.
- Other surgical approaches.

Table 2. Stages of Dry Eye Treatment

Treatment algorithms are often built to recommend a sequence of treatments according to the stage of the disease, but this is not possible for dry eye, as it is a complex condition that varies from patient to patient in both severity and in character. However, with the intention of helping ophthalmologists to create a logical and evidence-based treatment approach, the treatment algorithm shown in Table 2 was proposed within the framework of the TFOS DEWS II. For patients who do not respond to a certain level,

or who show greater severity, the next level is recommended, and in some cases, previous treatment may continue, in addition to any new treatment. In general, the approach is to start with conventional low-risk and normally available treatments, such as eye lubricants for the early stages of the disease, and progress to more advanced treatments for more severe clinical conditions.
The characteristics of the tear film and the hydrodynamics of the lacrimal system mean that ophthalmic formulations have a short retention period on the ocular surface, which is why frequent application is necessary to achieve the appropriate concentration of the substance used. [19]

Pharmacokinetic studies conducted in 12 patients with dry eye showed that the 0.2% SH solution had a mean permanence on the ocular surface of 11.1 minutes, while for the 0.3% SH solution it was 23.5 minutes, these solutions were eliminated from the ocular surface in approximately 45 minutes through the lacrimal system. [20]

Benzalkonium chloride is the most commonly used preservative in ophthalmic multi-dose products. [21] It is a bactericidal and antifungal agent that helps minimize the growth of microorganisms in multi-dose containers, [21] however, there is scientific evidence supported by *in vitro, in vivo,* and clinical models of the disruptive behavior of benzalkonium chloride in lacrimal stability, it is also the cause of cell damage in the corneal and conjunctival epithelium, inducer of changes due to inflammation and generator of a decrease in the function of the corneal epithelial barrier. [2] [22]

With the development of new technologies such as the Novelia bottle used in Multi-dose Lagricel® Ofteno, it has been possible to create a new generation of preservative-free products in multi-dose presentation at an affordable cost. The Preservative-Free system of the Novelia bottle has been shown to be able to protect the contents from contamination, which adds safety to the use of the drug by avoiding the addition of preservatives. [23] [24]
Laboratorios Sophia S.A. de C.V. carried out a phase I clinical trial in 2019, to evaluate the safety and tolerability of the Multi-dose Lagricel® Ofteno ophthalmic solution compared to Single-dose Lagricel® Ofteno. A total of 34 healthy subjects were evaluated in two parallel groups, demonstrating that both products are safe and tolerable. [25]

Currently in Mexico, for ophthalmic drugs that change their presentation, it is not necessary to carry out clinical studies; Laboratory studies, in which it is shown that the bottle does not affect the solution, is sufficient.

However, the commitment acquired with quality by Laboratorios Sophia, S.A. de C.V. drives it to exceed the minimum requirements for its products, so it seeks to demonstrate the efficacy of Multi-dose Lagricel® Ofteno in a phase II study.

This study will be carried out in a population with a diagnosis of mild to moderate dry eye, and will evaluate the efficacy of 3 doses of Multi-dose Lagricel® Ofteno (0.4% sodium hyaluronate).

2.2 Investigation product background

2.2.1 Pharmacology

SH is a glycosaminoglycan with viscoelastic rheology. It is made up of repeated units of N-acetyl-D-glucosamine and sodium-D-glucuronate. [26]

SH has a great capacity to retain water, this is one of its characteristics why it is present in a large number of artificial tear formulations, as it improves eye hydration, decreases surface friction of the eye and has excellent biocompatibility. [8] [27]

SH derived from Hyaluronic Acid shares many of its beneficial properties with the latter. In addition to water retention, which makes it a good moisturizer and lubricant, SH is hypoosmolar. This last characteristic allows it to decrease the osmolarity of the tear and mucinoid filaments. [28]

2.2.2 Efficacy

The efficacy of SH 0.15% in the treatment of patients with dry eye syndrome has been determined, using 1 drop every 4 hours in each eye for 30 days, it was shown that this pharmacological agent decreases inflammatory markers expressed by surface cells corneal (macrophages and T lymphocytes) and restores the tear film, resulting in a decrease in corneal inflammation. [29] It can be postulated that by decreasing inflammation of the ocular surface, there would be greater density of goblet cells in the conjunctiva and less apoptosis in corneal cells. [8]

In another clinical trial, the efficacy of SH 0.18% in the treatment of dry eye was evaluated and it was shown to be more efficient than carboxymethylcellulose 1%. Using fluorescein staining as a marker of corneal status, SH was found to restore the corneal surface faster and decrease dry eye symptoms. [30]

Hyo, L et al., evaluated the efficacy of 0.1% and 0.18% SH formulations in the treatment of dry eye. The study involved 30 patients with mild dry eye syndrome (1st 3 groups) and 30 with moderate dry eye (Group 4 to 6):

- Group 1. 15 patients who received SH, 0.1% (300 mOsm/l) free of isotonic preservative, 1 drop 4 times a day for 90 days.
- Group 2. 15 patients who received SH 0.18% (150 mOsm/l) hypotonic preservative-free 1 drop 4 times daily for 90 days.
- Group 3. 15 patients exposed to SH 0.1% isotonic (1 drop 4 times daily) + fluorometholone 0.1% (2 times daily) + cyclosporine 0.05% (2 times daily).
- Group 4. 15 patients exposed to hypotonic SH 0.18% (1 drop 4 times daily) + fluorometholone 0.1% (2 times daily) + cyclosporine 0.05% (2 times daily).

Hyo's results showed that the use of 0.1% and 0.18% SH is equally effective for the treatment of mild to moderate dry eye syndrome at the end of 90 days of intervention, considering the stability of tear film and improvement of the corneal surface. [31]

2.2.3 Safety

The safety of Lagricel® Ofteno has been evaluated in several clinical studies. A phase II clinical study evaluated its safety and efficacy, compared to 0.2% polyacrylic acid gel (Viscotears®), after LASIK surgery. This was a prospective, randomized, controlled study that included 30 patients, who received the study drug three times daily (TID) for 28 days. Outcome variables included: red eye, foreign body sensation, dryness, pain, photophobia, CVB, conjunctival hyperemia, corneal surface integrity, corneal opacity, Bengal rose staining, and findings in the periocular area and fundus. In this study, the safety and efficacy of Lagricel® Ofteno were tested. [32]

Another randomized, controlled, crossover clinical trial in 20 patients tested for efficacy in reducing signs and symptoms (e.g., red eye, foreign body sensation, and dryness) associated with dry eye disease. [33]

A phase IV clinical study was recently concluded in which the effect of bromfenac 0.09% ophthalmic solution on conjunctival hyperemia in patients with pterygium, administered twice daily, compared to placebo, was evaluated.

Both groups used Lagricel® Ofteno TID concomitantly. The results demonstrated the efficacy of both arms in reducing hyperemia and symptoms associated with pterygium, being statistically significant when compared to the initial values.

In turn, the bromfenac group was statistically superior to the placebo group. No AEs related to Lagricel® Ofteno were reported. [34]

2.2.4 Summary of pharmaceutical development

Multi-dose Lagricel® Ofteno has been developed by Laboratorios Sophia, S.A. de C.V. It has the physicochemical characterization and the protocol of accelerated and long-term stability. The formulation is identical to that of Single-dose Lagricel® Ofteno with a Multi-dose presentation.

2.3. Research Background

2.3.1. Research question

There is no prior information on the efficacy of Multi-dose Lagricel® Ofteno in clinical studies. However, the efficacy and tolerability of Single-dose Lagricel® Ofteno has previously been evaluated in two single-center clinical studies. [32, 34, 33, 34].

In the two clinical studies of Single-dose Lagricel® Ofteno, TFRT, comfort, blurred vision, and the incidence of AEs were evaluated as outcome variables. Lagricel® Ofteno single-dose was well tolerated and the final results were statistically significant when compared with baseline values.

2.4. Risk-Benefit Evaluation

2.4.1. Potential known risks

Eye lubricants are safe formulations. SH has a known safety profile. The diagnostic tests considered in the study design are also considered safe.

It is only anticipated with the use of ophthalmic applications: burning, foreign body sensation and blurred vision. These are of mild intensity and transient, with a post-instillation duration of no more than one minute.

2.4.2. Known potential benefits

As subjects with a diagnosis of mild to moderate dry eye, they are expected to present improvement in ocular comfort in at least 1 of the dosages used during this study.

The benefits of the study will be to document the efficacy and establish the ideal dosage of Multi-dose

Lagricel® Ofteno.

2.5. Statement of the problem

Dry eye is a common disease, whose estimated prevalence varies widely depending on its classification. Although the pathogenesis of dry eye is multiple, the different treatment phases have as a common denominator the use of ocular lubricants.

There is a wide variety of topical lubricants; however, there is no evidence that one is better than another.

Ocular lubricants have not been shown to be sufficient to completely resolve the alteration of the ocular surface and inflammation observed in patients with dry eye, they have been shown to be effective in providing protection to the ocular surface and reducing symptoms and clinical findings.

Multi-dose Lagricel® Ofteno obtains from SH its viscoelastic, hypoosmolar and water retention properties, to function as an effective lubricant that protects the ocular surface and reconstitutes the tear film, without the association to a preservative.

2.5.1. Justification

Dry eye is defined as a multifactorial disease of the ocular surface. The tear film plays an essential role in lubricating and protecting the ocular surface, as well as optimal visual performance.

Patients with dry eye, regardless of their etiology and degree of severity, will have to use eye lubricants to reduce symptoms and improve their quality of life.

Ocular lubricants are the first line of treatment for eye symptoms related to dry eye in subjects who refer to them healthy, with a prevalence of 5 to 35% in people over 50 years of age. [35]

If we add patients with occasional symptoms or who depend on a work or occupational situation and who will use it for intermittent periods throughout their lives, the spectrum of the population that will have access to these drugs is very extensive.

It is estimated that 50% of patients diagnosed with dry eye without comorbidities will use more than 2 types of ophthalmic solutions in 5 years of treatment.

This is why preservative-free lubricants have gained importance, to reduce the deleterious effect of the accumulated dose of preservatives.

One of the disadvantages of preservative-free ophthalmic products is the single-dose presentation, which

makes the treatment more expensive for various reasons, including product shrinkage. With the development of new technologies, it has been possible to create a new generation of preservative-free products in multi-dose presentation.

Multi-dose Lagricel® Ofteno is a preservative-free lubricant in multi-dose presentation of which it is necessary to document its efficacy and determine its ideal dosage.

3. Objectives and hypotheses

3.1 Main objective

- To assess the efficacy of 3 dosages of Multi-dose Lagricel® Ofteno in patients with mild to moderate dry eye by improving OSDI score.

3.2 Specific objective

- To assess the safety of 3 doses of Multi-dose Lagricel® Ofteno in patients with mild to moderate dry eye by means of the incidence of unexpected AEs related to the investigational product.

3.3 Secondary objectives

- To determine the efficacy of the multi-dose formulation of Lagricel® Ofteno by changes in the TFRT in twice-daily (BID), four-times-daily (QID), and six-times-daily (6x/D) dosages.
- To determine the efficacy of the formulation of Multi-dose Lagricel® Ofteno by changing corneal and conjunctival stains with lissamine green in the BID, QID and 6x/D dosages.
- To determine the efficacy of the multi-dose formulation of Lagricel® Ofteno by changing corneal and conjunctival stains with fluorescein in the BID, QID and 6x/D dosages.
- To determine the efficacy of the multi-dose formulation of Lagricel® Ofteno by changes in conjunctival hyperemia in the BID, QID and 6x/D dosages.
- To determine the efficacy of the multi-dose formulation of Lagricel® Ofteno by the incidence of chemosis at the BID, QID and 6x/D dosages.
- To determine the safety of the multi-dose formulation of Lagricel® Ofteno by changes in BCVA at BID, QID, and 6x/D dosages.
- To assess the safety of the multi-dose formulation of Lagricel® Ofteno by changes in IOP at BID, QID, and 6x/D dosages.

3.4 Hypotheses

H_0: Multi-dose Lagricel® Ofteno decreases the OSDI test score by 20% or more at the end of the study in at least 1 of the proposed dosages.

H_1: Multi-dose Lagricel® Ofteno does not decrease the OSDI test score by 20% at the end of the study in at least 1 of the proposed dosages.

4. Study design

4.1 Study overview

Phase II, multicenter, comparative, controlled, parallel-group, open-label, randomized clinical trial.

Justification of the study design.

4.2 Justification of the study design

The design of the study (clinical trial) is considered the highest standard of data quality when seeking to explore the effect of an intervention. The drug development phase (phase II) corresponds to the objective of the study, which is to evaluate efficacy and ideal dosage. The presence of parallel groups allows comparison between intervention groups on outcome variables. Due to the nature of the design, blinding was not considered for this study.

4.3 Expected duration

The total duration of the study, from the first visit of the first patient to the preparation of the final report, is estimated to be 7 months.

The planned recruitment period is 5 months, however, as 1 month of treatment is required, the final recruitment period will be 4 months. Considering that the proposed sample is 141 subjects, the total average recruitment rate during the study should be no less than 1 subject per day. This may vary depending on the number of open centers.

The approximate duration of each subject in the study is up to 34 days.

5. Study population

5.1 Eligibility criteria

5.1.1 Inclusion criteria

- Be of legal age.
- Have the ability to voluntarily give their signed informed consent.
- Be able and willing to comply with scheduled visits, treatment plan, and other study procedures.
- Be willing to modify lifestyle activities.
- Women of childbearing potential must ensure the continuation (initiated ≥ 30 days prior to signing the ICF) of the use of a hormonal contraceptive method or IUD during the study period.
- Have a diagnosis of mild to moderate dry eye, defined by:
- OSDI from 13 to 32 points
- + 1 of the following:
    - Corneal staining with more than 5 sites.
    - Conjunctival staining with more than 9 sites.
    - TFRT < 10 seconds.

5.2 Criteria for exclusion and substitution of subjects

5.2.1 Exclusion criteria

- Be pregnant, breastfeeding, or planning to become pregnant within the study period.
- Have participated in another clinical research study ≤ 30 days prior to the scrutiny.
- Have previously participated in this study.
- Have a better corrected visual acuity (BCVA) of 20/200 or worse in one of the eyes.
- Present an ophthalmological diagnosis of:
    - Allergic, viral, or bacterial conjunctivitis.
    - Anterior blepharitis.
    - Parasitic eye infections (e.g., Demodex).
    - Unresolved eye trauma.
    - Healing diseases of the ocular surface.
    - Corneal or conjunctival ulcers.
    - Filamentous keratitis.

- Neurotrophic keratitis.
- Bullous keratopathy.
- Neoplastic diseases on the ocular surface or adnexa.
- Diseases with fibrovascular proliferations on the conjunctival and/or corneal surface.
- Retinal and/or posterior segment diseases that require treatment or threaten visual prognosis.
- Glaucoma.

– Have a management of your dry eye that requires the implementation of stage 2 treatments of the TFO DEWS II recommendations in the treatment and staged management for dry eye disease.
– Have a history of drug addiction or drug dependence current or within the last two years prior to signing the ICF.
– Have a history of eye surgical procedure within the last 3 months prior to signing the ICF.
– Be a soft or hard contact lens wearer. You will be able to enter if you can suspend its use during the study, you must complete 15 days without using the contact lens prior to your inclusion.
– Have another medical condition, acute or chronic, that in the investigator's judgment may increase the risk associated with participation in the study or administration of the investigational product, or that may interfere with the interpretation of the results of the study.
– Have known hypersensitivity to the components of the investigational products.

5.2.2 Subject substitution

The sponsor, with prior authorization from the Research Ethics Committees (REC), may decide to replace the subjects who withdraw their ICF or those who present loss of follow-up, in case it is necessary to balance the groups of studies so that they are evaluable.

5.3 Lifestyle considerations

For the study, participants may need to modify some lifestyle activities to comply with the following:

- Abstain from tobacco use.

- Refrain from using electronic vaporizers.
- Avoid submerging yourself in water without eye protection (*goggles*).
- Avoid direct exposure to fans (including air conditioning vents) during activities that involve vision. 24 hours before your check-up visits.
- Maintain your sleep-wake cycle with which you enter the study.

5.4 Screening failure

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

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

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

Subjects who do not meet the eligibility criteria to participate in the study due to a specific modifiable factor may re-participate in the scrutiny. The subjects in this case must use the same initial count number.

5.5 Recruitment and retention strategies

This is a phase II study, which is planned to be conducted at least 4 centers. The selected centers will be responsible for the recruitment of the subjects.

The minimum expected recruitment rate is 1 subject per day.

The duration of the subject's participation in the study is approximately 33 days, during which time he or she will only have to attend two visits after the initial one. The subjects will be entitled to travel support for transportation and for complying with their visits. Other strategies to improve subject retention include:

- Clearly inform the importance of the study and the benefits that the population will obtain from its results.

- Make calls or send text messages to remind you of appointments or activities to do.
- Provide a printed calendar and an identification card in order to remember appointments and activities that will be carried out, in addition to the estimated duration of the same.
- Offer flexible hours of operation.
- Systematic organization of the study procedures, so that the subject does not last longer than necessary in his visit.
- Minimize subject wait times.

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

5.6 Procedure in case of loss of follow-up

For this protocol, loss of follow-up is defined as those subjects who were randomized, who at some point were active subjects of the study, but their final evaluation could not be completed.

In the event that the participating subject does not attend their appointment, the research center must make a call to find out the reason and will try to make a new appointment within the established window period or an unscheduled appointment. If it is not possible to make an appointment, the presence of AEs and the reason for leaving the study will be asked as minimum data.

A loss of follow-up <20% is considered not to be a problem for the validity of the results obtained. [36, 37]

5.7 Subject identification

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

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

Example:

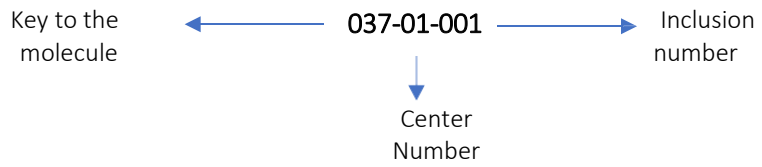
A.	Arieh Daniel Mercado Carrizalez	B. Juan de la Torre Orozco
a.	Initials: AMC	b. Initials: JDO

In the scrutiny stage (within the basal visit (BV)) the participant number will be assigned using 3 consecutive digits. Once the subject has been selected, they will be assigned a number with which

they will be identified throughout the study. This code will be made up of eight numbers in the following order from left to right:

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

Example:



6. Investigational product

6.1 Administered product

1. Investigational product

- Generic name: Sodium hyaluronate.
- Distinctive name: Multi-doses Lagricel® Ofteno.
- Active ingredients: Sodium hyaluronate 0.4%.

[redacted]

- Pharmaceutical form: Ophthalmic solution.
- Presentation: Multi-dose dropper bottle.
- Prepared by: Laboratorios Sophia, S.A. de C.V.
- Solution Description: Transparent solution, free of visible particles.
- Packaging description: White bottle with a capacity of 10 mL, made of low-density polyethylene.

2. Dose of the investigational medicinal product

Three different dosages will be used, each of the groups will use one of the following:

- One drop BID, OU, for 30 days (suggested time between applications is 12 hours).
- One drop QID, OU, for 30 days (minimum time between applications of 3 hours).
- One drop 6x/D, OU, for 30 days (minimum time between applications of 3 hours).

6.1.2.1 Dose Justification

Generally, ophthalmic lubricants are prescribed as needed (*pro re nata* [PRN]). [38] However, the study by Asbell *et al* compared the efficacy of QID dosage against PRN. Their results showed that, although there was no difference in clinical signs, the QID group had greater improvement in symptomatology. [39]

For these reasons, 3 different dosages are proposed for this study, considering that the optimal dosage of IP would be QID.

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

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

The reception will be carried out by the assigned personnel of the research team. The good condition of the primary packaging (box) must be verified.

In the event that you show alterations or defects in its integrity that in your judgment could have damaged the content, you must report it to the sponsor. If the package does not show significant defects, it will proceed to open it.

Inside, the acknowledgment of receipt document and the temperature *data logger* must be located.

It must be checked that the temperature recorded complies with what is specified for its transport and safekeeping. The content (IP) will be verified with what is reported in the document. In case the document corresponds to the content, you will sign the receipt and send it to the sponsor. Otherwise, the sponsor will be notified.

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

The storage temperature should be no more than 30°C.

The research center is obliged to record, in the designated format, the temperature recorded in the *data logger*, every day while the protocol is in force and has IPs. These data will be reviewed by the clinical monitor according to the record in the *data logger.*

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

Upon completion of the protocol, all study material will be retrieved by the sponsor as part of the closing audit.

The final delivery of material will be made by the principal investigator or the person designated by him to deliver material at the end of the study.

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

6.3 Concomitant treatments and medications not authorized during the study

Unauthorized medicines will not be allowed during the intervention period, including the period between the end of BV and D1, from D1 the IP application scheme will begin. The objective of this restriction is to avoid drug interactions that could alter the results of the variables evaluated.

Medications allowed: Ophthalmic: Tetracaine 0.5%, Tropicamide 0.8% / Phenylephrine 5% (for study procedures) and IP. Non-ophthalmic: Hormonal contraceptives. At the discretion of the investigator, systemic medicines whose effects do not modify the efficacy or safety parameters of this protocol may be used, but must be reported to the sponsor's scientific committee to determine the participant's status as appropriate.

Prohibited Drugs: Any ophthalmic drugs that are not on the list of allowed drugs. Systemic medications: steroids, immunomodulators, and tetracyclines.

6.4 Procedure for monitoring and measuring adherence

For more than four decades, research has been conducted on the appropriate way to measure and quantify medication adherence, but none is considered the gold standard in both cross-sectional and longitudinal studies. [40, 41, 42, 43, 44, 45, 46, 47] There are different procedures to measure adherence to pharmacological interventions. The most common procedure is self-reporting, which includes: patient interviews, questionnaires and self-monitoring diaries. Its strengths are speed, flexibility, low cost and ease of implementation; They have a high degree of specificity for non-adherence, but the sensitivity and reliability for adherence is low. [47, 48] The biochemical measurement of the drug, or its metabolite, are methods that better confirm the use of the drug; but it raises costs, is impractical and of little use in ophthalmic applications, since concentrations at the peripheral level could be undetectable; samples of other tissues involve more invasive methods that would not be advisable. [47]

Medication counting is another way to measure adherence. Classically referred to as "pill counting," in ophthalmology it translates to the weight of the bottle. This is a simple, inexpensive, and non-invasive method.

The main disadvantages of this method are:

1. The application of the medication cannot be confirmed (it may have been intentionally pulled or instilled outside the eye)
2. It depends on the subject bringing back the medication. [47, 48]

However, in this study, the initial and final weight of the bottle will also be taken into account to measure adherence, at the same time adherence will be measured through the subject's diary.

A multi-procedure approach to measuring adherence is recommended. Because there is no measurement of ideal adherence, it is appropriate to use more than one method when trying to achieve results that resemble reality. Selecting two or more methods allows their strengths and weaknesses to be compensated, to capture adherence levels more accurately. [46]

The evaluation of adherence will be favored by means of the weight of the bottle and will be carried out in the following way, taking into account the following information: the weight of the drop, the initial weight of the container, the final weight of the container and the calculation of the total applications. The following simplified formula shall be used:

$$Ad = \frac{(Pi - P_f)100}{PT}$$

Where:

Ad = adherence

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

Pf = weight of the container returned by the subject

PT = weight of the dosage indicated for the intervention

$$PT = (Pg)G$$

Where:

Pg = weight of one drop of the intervention, determined by the research and development department

G = number of applications indicated for the intervention

Packaging that does not retain its physical integrity will not be considered for the calculation of adherence. In cases where the container is not returned, or it has not preserved its physical integrity, adherence will be measured only through the subject's diary

Adherence through the subject's diary will be measured as follows:

$$Ad = (Ar)100/Ai$$

Ad = Adhesion

Ar = Registered Applications

Ai = Applications indicated for the products under investigation

There is no standardized parameter to define adequate adherence, it must be defined and delineated by the objectives of the particular research. [47]

For this study, a minimum adherence of 70% will be considered to be what is necessary to meet the objectives of the research both by subject diary and by bottle weight. Therefore, subjects who have an adherence of less than 70% will not be considered for the efficacy analysis, they will only enter the safety analysis.

6.5 Strategy to improve adherence

1. The Principal Investigator (PI) will sensitize the subject to the importance of the correct application of the research product (IP) to achieve the objectives of the study.
2. Direct questioning by the PI about the application of the IP.
3. Delivery of a printed calendar specifying the date of the visit and its activities.
4. Training in the filling and revision of the subject's diary.
5. If deemed necessary, text messages may be sent as reminders. The content of these messages must be previously approved by the REC.

7. Methods and procedures of the study

This study will be carried out in research centers previously evaluated by the sponsor. These centers will be institutions or establishments where health research is carried out that complies with current regulations.

7.1 From the research center

According to the capacities of the centers evaluated during the feasibility, the number of centers that will be included will be determined. It is suggested that there are 4 or more.

The research center will be responsible for forming a multidisciplinary research team to execute the clinical study according to protocol. It is their prerogative to design the organization and select the personnel who will perform the functions. However, it is the sponsor's need for the PI to be a specialist in Ophthalmology.

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

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

The sponsor must ensure that all study site personnel participating in the study are adequately trained on the study (research protocol, investigator's manual, amendments, standard operating procedures, etc.) and on the good practices of the study.
Clinical Internships of the International Conference on Harmonization (ICH), before the start of their participation in it. Training must be recorded in writing and those records must be filed in the master record of the study.

7.2 Clinical study registration

This clinical study will be registered by the sponsor in public clinical trial registries before its start (inclusion of the first patient): National Registry of Clinical Trials (RNEC) of the Federal Commission for the Protection against Sanitary Risks (COFEPRIS) and in a primary registry platform of the World

Health Organization (WHO). WHO Primary Registries meet specific criteria for content, quality and validity, accessibility, unique identification, technical capacity and administration. WHO Primary Registries meet the requirements of the International Committee of Medical Journal Editors (ICMJE).

7.3 Randomization

Randomization of subjects will be carried out using a computer-based assignment system. After signing the ICF, the patient will receive a patient number with which all their information will be encoded pseudonymity during collection and completely anonymous during analysis.

The generation will be carried out by a third party, authorized by Laboratorios Sophia, S.A. de C.V., through its electronic system. The information pertaining to this third party will be on file.

Although it is an open-label study, a secondary packaging masking will be performed.

The IP will be identified by means of labels in accordance with current and applicable regulations, which must contain at least:

- Sponsor's name, address, and phone number.
- Dosage form and route of administration.
- Batch number.
- Legend "For clinical studies only"
- Expiration date.

7.4 Outcome variables

7.4.1 Primary outcome of efficacy

- OSDI test score (ET: day 31).

7.4.2 Primary outcome of safety

- Incidence of AEs (ET: days 15 and 31).

7.4.3 Secondary outcome of efficacy

- Changes in the TFRT (ET: day 15 and 31).
- Corneal and conjunctival staining changes with lissamine green (ET:15 and 31).
- Corneal and conjunctival staining changes with fluorescein (ET: days 15 and 31).
- Conjunctival hyperemia changes (ET: day 15 and 31).
- Incidence of chemosis (ET: days 15 and 31).

7.4.4 Secondary outcome of safety

- Changes in the BCVA (ET: day 15 and 31).

- Changes in IOP (ET: day 15 and 31).

7.4.5 Definition of variables, methods and scales to be used for measurement

Variable	Type of variable	Unit (symbol)	Method of measurement	Normal value	Evaluation time	Test statistics
Primary outcome of efficacy						
OSDI	Quantitative discrete	Score	Questionnaire	< 13	BV and FV	ANOVA Student's t test*
Secondary outcome of efficacy						
TFRT with fluorescein	Quantitative continuous	Second(s)	Direct observation with slit lamp and cobalt blue filter	> 10s	BV, V1 and FV	ANOVA Student's t test*
Corneal and conjunctival staining with lissamine green	Categorical ordinal	Degrees	Direct observation with slit lamp, evaluated with Oxford's scale	0	BV, V1 and FV	Pearson's χ^2 or Fisher's exact test. McNemar's test*
Corneal and conjunctival staining with fluorescein	Categorical ordinal	Degrees	Direct observation with slit lamp, evaluated with Oxford's scale	0	BV, V1 and FV	Pearson's χ^2 or Fisher's exact test. McNemar's test*
Conjunctival hyperemia	Categorical ordinal	Grades (Efron)	Direct observation with slit lamp, evaluated with Efron's scale	0	BV, V1 and FV	Pearson's χ^2 or Fisher's exact test. McNemar's test*
Chemosis	Categorical nominal	Present/absent	Observation	Absent	BV, V1 and FV	Pearson's χ^2 or Fisher's exact test. McNemar's test*
Primary outcome of safety						
AE	Quantitative discrete	Number of Cases (N)	Count	0	BV, V1 and FV	ANOVA
AE(bis)	Categorical nominal	Present/absent (-)	Observation	Absent	BV, V1 and FV	Pearson's χ^2 or Fisher's exact test.
BCVA	Quantitative discrete	Fraction (-)	Snellen chart	1	BV, V1 and FV	ANOVA Student's t test*
IOP	Quantitative discrete	Number	Tonometry	≥10 and ≤21	V1 and FV	ANOVA Student's t test*

OSDI, ocular surface disease index; **TFRT,** tear film rupture time; **AEs,** adverse events; **BCVA,** better corrected visual acuity; **IOP,** intraocular pressure; **ANOVA,** Analysis of Variance; X^2, Chi-square. ***When applicable.**

Table 3. Operational definition of variables

The variables, method, and scales for their measurement are described in detail below. They are in order according to Table 3.

7.4.5.1 OSDI Score

The OSDI is a validated and reliable instrument to measure the severity of dry eye, it has the necessary psychometric properties to be used as an outcome variable in clinical studies. [49] It consists of a questionnaire with 3 questions and 12 items, the answer is in frequency and offers 5 options from 4 (at all times) to 0 (at no time), questions 2 and 3 also contain the NA option. (See: 16.1) The OSDI score is calculated based on the following formula:

$$OSDI = \frac{D \times 25}{E}$$

Where:

D = sum of the points of the questions answered

E = number of items answered, those answered with NA are not counted as answered

The OSDI score will be recorded on the electronic Case Report Form (eCRF).

Management as AE: An increase of more than 40% in the value of the OSDI score at the basal visit should be reported and managed as an AE for lack of effectiveness.

7.4.5.2 Tear film rupture time

One of the first aspects of the tear film to change 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 of assessing tear film stability is fluorescein evaluation of TFRT. Once the fluorescein has been instilled, the cobalt blue filter asks the patient not to blink after blinking 1 to 2 times. The fluorescein-colored precorneal layer will change to less fluorescent or non-fluorescent regions. The time that elapses from the last blink to the appearance of these regions is the TFRT. It will be reported in seconds, in the clinical record and in the eCRF.

Management as AE: a decrease in TFRT of more than 40% compared to baseline should be reported and managed as an AE due to lack of efficacy.

7.4.5.3 Corneal and conjunctival staining with fluorescein

A drop of topical anesthetic will be instilled in the bottom of the conjunctival sac, then a second drop will be applied to the tip of the fluorescein strip, allowing it to sit on the strip for 5 seconds to elute the dye, shaking off the excess at the end. A small contact of the strip is made with the conjunctiva at the fundus of the temporal sac, while the patient looks upwards, without damaging the conjunctiva. It will be graded according to the Oxford scale (See: 16.2). [50]

The PI will record in the file and the eCRF the grade awarded for fluorescein staining of DO and OS, respectively.

Management as AE: corneal stains that are equal to or greater than grade III will be considered as AEs.

7.4.5.4 Corneal and conjunctival staining with lissamine green

After the revision with fluorescein, a drop of saline solution will be applied to the tip of the green lissamine strip, allowing it to sit on the strip for 5 seconds to elute the dye. A drop of the strip is instilled at the bottom of the temporal sac, while the patient looks upwards, without damaging the conjunctiva. The patient may be asked to blink repeatedly to prevent accumulations in the conjunctival folds. The examination should be done between 1 and 4 minutes after instillation through a neutral density filter or with the red-free filter. It will be graded according to the Oxford scale. [50]

The PI will record in the file and the eCRF the grade awarded for fluorescein staining of DO and OS, respectively.

Management as AE: corneal stains that are equal to or greater than grade III will be considered as AEs.

7.4.5.5 Conjunctival hyperemia

Conjunctival hyperemia is defined as the simplest reaction of the conjunctiva to a stimulus, with a red appearance secondary to vasodilation of the conjunctival vessels of variable intensity. You will graduate using the Efron scale (See 16.3). [51]

Management as AE: conjunctival hyperemia classified as grade 3 or higher will be considered as AE.

7.4.5.6 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 whether the conjunctiva separates from the sclera by ≥ 1/3 of the total eyelid opening or if it exceeds the gray line. [52]

Management as an AE: your presence will be considered as an AE.

7.4.5.7 Adverse events

As defined in section 8.1.1, an AE is any unfavorable medical occurrence in a subject to whom an IP is administered, regardless of causal attribution.

Management of AEs will be performed as described in the Adverse Events section.

The PI will record in the corresponding section of the eCRF the AEs that the study subjects present, in addition to referring them to the clinical file.

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

The AEs expected for the use of PIs are: blurred vision, burning, eye irritation, foreign body sensation, and sticky eyelash sensation. They are expected to be transient, lasting no more than 30 seconds after IP instillation, and to be of mild intensity.

7.4.5.8 Better corrected visual acuity

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

Snellen's notation is described as the distance at which the test is performed, divided by the distance at which the letter is vertically equivalent to 5 arc minutes. Thus, at 6 meters a letter 6/6 (20/20) is equivalent to 5 minutes of arc, a letter 6/12 (20/40) is equivalent to 10 minutes, and a letter 6/60 (20/200) is equivalent to 50 minutes. The Snellen fraction can also be expressed as a decimal (i.e., 20/20 = 1 and 20/40 = 0.5). [53]

VA will be evaluated at baseline, without refractive correction with the Snellen chart. It 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 the right eye (OD) 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 can see will be noted by the evaluator in fraction as the VA of OD in the clinical record. The OS is proceeded with the same method.

Subsequently, the subject's objective and subjective refractive correction will be performed. The result of the subjective refraction will be reported as CVMA, it will be noted in fraction in the clinical record and in the eCRF it will be noted in decimal. By definition, BCVA cannot be inferior to VA.

Management as AE: A decrease of more than 2 lines on the Snellen card must be reported and handled as AE.

7.4.5.9 Intraocular pressure

Intraocular pressure (IOP) is a measurement through tonometry, based mostly on the force required to flatten the cornea, or the degree of corneal indentation produced by a fixed force. Goldman tonometry is based on the Imbert-Fick principle. [53]

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

Management as AE: intraocular pressure equal to or greater than 22 mm Hg should be reported and managed as AE.

7.4.6 Study visits and activity programs

7.4.6.1 Description of activities per visit

The procedures are listed in the order in which they are suggested, trying to maintain the coherence of the evaluations and as far as possible, from the least invasive to the most invasive

7.4.6.2 Basal Visit

- Signature of the CRF: refers to the signing of the written informed consent document. Without obtaining informed consent, it is not possible to perform any of the study procedures
- Clinical history: refers to the technical, clinical, and legal document in which the subject's health conditions, medical acts, and other procedures performed on the subject are chronologically recorded. It includes anthropometric measurements, anamnesis, comprehensive ophthalmological examination that allows discerning the patient's eligibility, that is, evaluation of both eyes of ocular adnexa, slit lamp examination of the ocular surface and anterior segment and fundoscopy. If the subject is taken from the established population base of the study center, the existing medical history may be used, and only an update must be made.
- Evaluation of concomitant medications: refers to the interrogation by the PI of the subject, inquiring about the use of medications.
- Urine pregnancy test: This refers to performing a rapid pregnancy test on all women of childbearing potential who wish to enter the study. By fertile age we mean women who have had their menarche and have not presented their menopause. Menopause is defined as 12 months from the last menstruation in women over 40 years of age, or who have had a hysterectomy or bilateral oophorectomy. Women of childbearing potential with contraceptive methods including bilateral tubal obstruction should be tested for pregnancy. This test will be performed by the PI or designated team person in accordance with the instructions on the device provided by the sponsor
- Vital signs: refers to the measurement of heart rate, respiratory rate, systemic blood pressure and temperature. This information must be contained in the patient's medical history and the evolution notes of the patient's clinical file.

- BCVA: See 7.4.5.8
- OSDI: See 7.4.5.1
- TFRT: See 7.4.5.2
- Ocular Surface Staining See 7.4.5.3 and 7.4.5.4
- Ophthalmologic evaluation: refers to the evaluation of the subject's ophthalmic structures, eyelids and adnexa, ocular surface, anterior segment, and posterior segment; not considered within the outcome variables. This evaluation is intended to identify alterations that may interfere with the course of the investigation or identify AEs. This examination will be recorded in the clinical file, in the CRF only what is considered an AE will be reported.
- IOP: See 7.4.5.9
- Eligibility criteria: refers to the review by the PI, where it is found that the subject can be included in the study by meeting the inclusion criteria and not meeting the exclusion criteria. See 5.1
- IP Assignment: This refers to determining the intervention that the patient will follow during the study. It will be carried out in accordance with section 7.3. This assignment will be made at the basal visit (day 1).

- Delivery of the IP and initiation of intervention: This refers to the delivery of the IP to the study patient, by the research center.
- AE Evaluation: Ver 7.4.5.7
- Delivery of patient material and filling instructions: This refers to the delivery by the PI to the subject of the subject's diary, identification card and calendar improving adherence. The assigned personnel will carry out prior training on the subject, on the filling of the diary.

7.4.6.3 Follow-up visit

- Concomitant Drug Evaluation: See 7.4.6.2
- Vital Signs: See 7.4.6.2
- BCVA: See 7.4.5.8
- Tear Film Rupture Time: See 7.4.5.2
- Ocular Surface Staining: See 7.4.5.3 and 7.4.5.4
- Comprehensive ophthalmologic evaluation: See 7.4.6.2
- IOP: 7.4.5.9
- AE Evaluation: Ver 7.4.5.7
- Adherence assessment: Adherence will be assessed in two ways, one through the initial *vs.* final weight of the medication bottle and the other through the record of applications in the subject's diary, adherence will be calculated. Adherence will be calculated based on the dosage of 2, 4 and 6 applications per day.

7.4.6.4 Final visit

- Concomitant Drug Evaluation: See 7.4.6.2
- Urine Pregnancy Test: See 7.4.6.2
- Vital Signs: See 7.4.6.2
- BCVA: See 7.4.5.8
- Tear Film Rupture Time: See 7.4.5.2
- Ocular Surface Staining: See 7.4.5.3 and 7.4.5.4
- Comprehensive ophthalmologic evaluation: See 7.4.6.2
- IOP: See 7.4.5.9
- AE Evaluation: Ver 7.4.5.7
- OSDI: See 7.4.6.2

- Evaluation of the subject's diary: Refers to the review of the subject's diary instrument by the PI or the staff of the designated center; it will be reviewed that it is correctly filled in, as well as the patient's comments, of these comments the PI may interrogate for AEs. At the discretion of the PI, comments may or may not be reported as AEs.
- Evaluation of adherence: See 7.4.6.4
- Return of IP and subject's diary: refers to the subject's return of the IP and the subject's diary to the research center.

7.4.6.5 Unscheduled follow-up visits

At the request of the patient or any other individual involved in the study, unscheduled follow-up visits may be conducted for the reporting of AEs. During these visits, all pertinent data on the AEs reported should be collected and, where appropriate, an appropriate management plan should be established.

7.4.7 Data collection

7.4.7.1 Source documents

Source documents are all written or printed records derived from automated processes (e.g., printouts of laboratory results issued by automated analysis equipment) where the information is recorded for the first time and that is part of the permanent records of the patient's history. Examples of source documents are the medical history, clinical evolution notes, laboratory reports, cabinet study reports, nursing notes, follow-up notes, surgery records, etc.

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

7.4.7.2 Electronic forms of data collection

All data related to the protocol will be captured through an eCRF by the investigation team staff. The data related to the protocol should NOT be captured directly in the eCRF, but should be transcribed from the corresponding source document. This procedure allows monitoring to be carried out for

verify the information captured in the eCRFs. It is the responsibility of the researcher to ensure that the information is transcribed into the eCRFs in a correct, complete, and timely manner. It is understood that all eCRF forms captured and submitted for data analysis are approved by the Investigator.

7.4.7.3 File

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

8. Evaluation and management of adverse events

8.1 Regulation and standards on adverse events

The registration and reporting of AEs will be carried out in accordance with the guidelines established in NOM-220-SSA1-2016, which is in accordance with the international guidelines ICH E6.

8.1.1 Meaning of adverse event

According to the ICH, an AE is any unfavorable medical appearance in a patient under clinical investigation who is administered a pharmaceutical product, regardless of causal attribution.

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

8.1.2 Relevant definitions to the classification of adverse events

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

Severity (mild, moderate, or severe). Mild are those that present with minimal symptoms, do not require treatment or suspension of the medication; moderate, when they interfere with usual activities, without threatening the patient's life, require treatment and may or may not require discontinuation of the medication; severe, those that interfere with usual activities and require pharmacological treatment and discontinuation of the medication.

Causality. It is the relationship that is assigned between the drug and the AE: certainly caused by the drug, there is clear evidence of causality, i.e. the AE reappears with the administration of the drug; probably caused by the drug, there is a high suspicion of causality but there is no direct evidence or it is considered unnecessary or dangerous, i.e. the reaction disappears when the drug is stopped; possibly caused by the drug, there is additional information to suggest that the cause may be due to

another drug or disease; unlikely to be caused by the drug, there is a clear explanation of the origin due to the underlying disease or the use of another drug; conditional, there is a lack of data to issue a clear causality; non-classifiable, those for which once all possible information has been obtained about the AEs, it remains unclassifiable.

8.1.3 Responsibilities of the researcher

It is the responsibility of the Investigator to verify AEs through questioning, review of the information recorded in the subject's diary, pertinent physical examination, assessment of evolution, as well as appropriate medical and pharmacological management; as well as to follow up until the resolution or outcome and definitive discharge of the AE, following the definitions determined in national and international regulations. [54] [55] [56]

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

The attention of the AEs will be carried out according to the event attention diagram (see Figure 5. Adverse Event Care).

In the final report to be written by the Clinical Team of the Medical Management of Laboratorios Sophia, S.A. de C.V., the report of AEs in compliance with current national and international regulations will be included. [55] [54]

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

8.1.4 Adverse event registration in the electronic case report form

AE registration considers:

- Subject identification information such as: subject number, age, gender, and if applicable

specify the eye.

- Information about the causality of the AE, its relationship to PIs, or to another study-related drug, as appropriate.
- Important date information:
    - Date on which the AE occurs.
    - Date on which the PI becomes aware of it.
    - Date of resolution or outcome, as applicable.
- Information on diagnosis and clinical management.
- If a lack of therapeutic response to PIs is detected, it must be reported as a serious AE within the period stipulated by current regulations. Include in concomitant medications the therapy used for the pharmacological management of the AE.
- Record the outcome or resolution of the event:
    - Recovered/resolved without sequelae
    - Recovered/resolved with sequelae
    - Not Recovered/Unresolved
    - Patient who died due to AEs
    - Patient who died and it is judged that the drug may have contributed
    - Patient who presented death and it is not related to the product or drug under investigation,
    - Unknown
- Information about the investigational product or drug or the drug associated with the AE, AMR, or SRAM. As applicable, the information concerning the generic name, distinctive name or code of the IP and/or investigational drug must be recorded, as appropriate according to the methodological design of the study, this is relevant in the case of blinded studies or in those where placebo is used as comparators, since there are circumstances that justify the opening of the blind to determine if the AE may be attributable to the active agent. the combination of active agents, or the pharmacologically inert substance(s), such as vehicles or additives, as appropriate to the phase of clinical research in which the development of the drug is located. It will also be necessary to include the data concerning the batch number, manufacturing laboratory, expiration date, dose, route of administration, start and end dates of administration and/or consumption, reason for prescription; according to whether it is an investigational product or drug (protocol in which the patient currently participates) or is a drug that the research subject consumes for the treatment of underlying concomitant

diseases or uses for the management of some transitory sign or symptom that does not correspond to the Natural History of the pathology that motivated his or her entry into the research protocol.

- Indicate the withdrawal or maintenance of the medication, as appropriate. Indicate whether the withdrawal of the IP or investigational drug or suspected drug (of causing the event) eliminates the AE. Also indicate if a dose adjustment is made, if the event changes in terms of intensity or seriousness, persistence of the reaction. It is important to indicate whether in those subjects who are exposed again to the drug, which had previously been discontinued, the AE reappears.
- Information regarding concomitant pharmacotherapy. Indicate the generic name, dose, route of administration, start and end dates of use, as well as the reason for the prescription regardless of whether it is in accordance with the information to prescribe or technical data sheet or is used outside the regulations or what has been authorized by the local, national or international regulatory entity.
- Relevant medical history information. The analysis of the AE considers the information previously narrated, despite the clinical context in which this harmful phenomenon occurs in the participants of the clinical research protocol, is of special interest, so the information about previous conditions, hypersensitivity or allergy phenomena, previous surgical procedures, laboratory analyses or cabinet examinations that have been performed on the participant, etc., that the researcher deems it appropriate to mention may do so.

8.1.5 Adverse event monitoring

The PI will provide the attention and follow-up of the AE presented by the participant until its outcome, according to what is referred to in the following section.

8.1.6 Serious adverse event procedure

The AE care process considers the following stages (Figure 5):

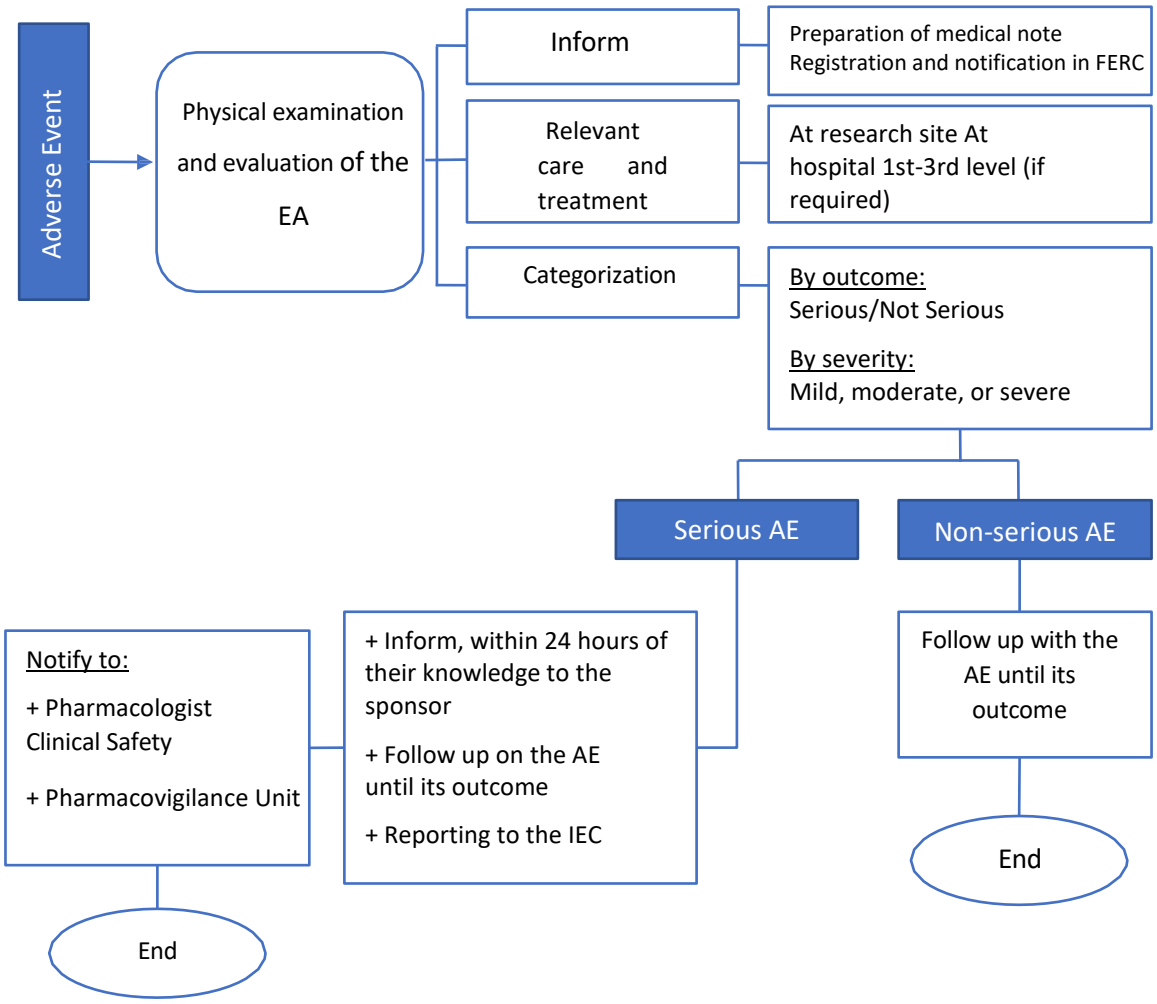


Figure 5. Adverse Event Care

During the development and conduct of this study, undesirable harmful events or adverse reactions of medical implication may occur in the research subject, which do not necessarily have a causal relationship with the PIs. These harmful phenomena can occur during the use of investigational drugs at doses authorized for use in humans by a local, national or international regulatory entity. However, it may be suspected that the IP or the investigational drug causes some unwanted clinical manifestation. AEs, ADRs or RAS to one or more 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, in such a way that:

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

to the knowledge of it, so that in turn it informs the Clinical Team and the UTFLS, and that it subsequently informs the REC. Non-serious AEs will be recorded and appropriately addressed and the safety profile of the IP or investigational drug will be reported to the appropriate regulatory entity in the final report of the clinical trial

The recording of the outcome of the AE depends substantially on the follow-up that the PI performs on the subject, since it is expected that most of the harmful phenomena (consult the investigator's manual) are ophthalmic in nature, however, there may be systemic alterations. Therefore, in the opinion of the researcher, the withdrawal of the participant or their permanence will be considered.

8.1.7 Causality evaluation

Causality assessment is the methodology used to estimate the probability of attributing the observed AE to a drug. It considers probabilistic categories according to the available evidence and the quality of the information, based on national pharmacovigilance regulations. [54]

The Pharmacovigilance and Technovigilance Unit of Laboratorios Sophia, S.A. de C.V. (UFTLS) can use the Karch and Lasagna algorithm modified by Naranjo referred to by Aramendi I, as a tool to facilitate the probabilistic categorization of causality. In this algorithm, different items are qualified, which allow assigning a value to the cause-effect relationship between the administration of the drug and the adverse reaction. [57] See Table 4. Karch and Lasagna's algorithm modified by Naranjo.

No.	Reagent	Score	
		Yes	**No**
1.	There are conclusive prior reports of 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 drug reaction, adverse event, or suspected adverse drug reaction improved by stopping or administering a specific antagonist	+1	0
4.	Adverse drug reaction/adverse event/suspected adverse drug reaction reappeared upon administration of the investigational drug/product/investigational drug	+2	-1
5.	There are alternative causes that can 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 fluids at toxic concentrations	+1	0
8.	The intensity of adverse reaction/adverse event/suspected adverse drug reaction was higher at higher doses or lower at lower doses	+1	0
9.	The patient has had similar reactions to the investigational drug/product or investigational drug in the past	+1	0
10.	Adverse reaction/adverse event/suspected adverse drug reaction confirmed with some objective evidence	+1	0
	Total score	Sum	
	Probabilistic category based on the score obtained		
I	The causal relationship is verified	≥.9	
II	The AMR is likely due to the investigational drug or product	5 to 8	
III	AMR may be due to the investigational drug or product	1 to 4	
IV	The causal relationship is doubtful	0	

Each item receives a defined score and the final sum allows estimating the probabilistic category of the cause-effect relationship between the administration of the investigational product and the adverse reaction, adverse event or suspected reaction Adverse.

Table 4. Karch and Lasagna's algorithm modified by Naranjo

In such a way, the degree of certainty to establish the IP as the causal agent of the harmful phenomenon that occurs to the subject of the clinical study. It can also be indicated directly

by the PI based on their clinical experience or through the voluntary application of the tool mentioned above. However, it is important for the researcher and the UFTLS to consider the following arguments in favor of causation:

a) Strength of association, which refers to the number of cases in relation to those exposed.
b) The consistency of the data, i.e., the presence of a common characteristic or pattern.
c) The exposure-effect pattern, which determines the relationship with the site of appearance, time, dose and reversibility after deletion.
d) Biological plausibility, which refers to the possible pharmacological or pathophysiological mechanisms involved in the development or presentation of the AE.
e) Experimental findings, e.g., the appearance of abnormal metabolites or high levels of the drug or its biotransformation product.
f) Analogy, which refers to the experience gained with other related drugs, adverse reactions frequently produced by the same family of pharmacological agents.
g) Nature and characteristics of the data, i.e., objectivity, accuracy and validity of the relevant documentation. [58]

8.1.8 Unanticipated problems

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

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

8.1.8.1 UAPs Report

The PI will be responsible for reporting UAPs to the sponsor, RC, REC. The report must contain the following information:

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

UAPs that are serious AEs must be reported to the IEC and the sponsor within the first 24 hours of the PI becoming aware of it.

Any other UAPs will be reported to the IEC and the sponsor within the first 5 business days after the PI becomes aware of it.

9. Study monitoring

The study sponsor is responsible for monitoring the study. Monitoring activities include, but are not limited to: general safety monitoring, general study quality monitoring, monitoring by study site, detection monitoring, reporting and tracking of AEs, monitoring for resolution of discrepancies in data capture, etc.

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

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

9.1 Monitoring of study centers

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

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

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

The final visit will take place at the end of the study, once the last participant in the last site has been discharged from follow-up. During this visit, the monitor will verify that the site has all the necessary documents to archive, that all biological samples have been sent for analysis, that all study drug (used and unused) has been recovered and sent to the sponsor, and that all unused material has been recovered.

Details on monitoring are set out in the corresponding plan.

9.2 Audit and quality control

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

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

9.3 Pre-study audit

Study sites included in the study will be subject to a pre-site feasibility visit, where they will be verified to meet the minimum requirements indicated by the sponsor.

9.4 Audit during the conduct of the study

They may take place at any time before, during, or after the conclusion of the study. If any audit or inspection is conducted, the investigator and the institution shall agree to allow the auditor/inspector direct access to all relevant documents, and shall allocate their time and that of their staff to the auditor/inspector to discuss the findings and any pertinent problems. In the event that the audit has not been scheduled by the sponsor, the facility must notify Laboratorios Sophia, S.A. de C.V. immediately.

10. Statistical analysis

10.1 Data analysis

10.1.1 Statistical analysis

The statistical analysis will be carried out by personnel of Laboratorios Sophia, S.A. de C.V. The statistical package SPSS version 19.0 for Windows (IBM Corporation, Armonk, NY, USA) will be used.

Although the study design is open-label, the personnel designated for the statistical management of the data will be blinded to the intervention groups to avoid possible interpretation biases. Coding will be performed using consecutive numbers for each intervention group.

The data will be collected and sorted in an Excel spreadsheet (Microsoft® Office). The data will then be exported to the SPSS package platform. The variables will be categorized according to their nature (see Table 3).

10.1.2 Data interpretation

The Kolmogorov-Smirnov and Shapiro Wilk tests will be performed, as appropriate, to determine if the distribution is normal in the results obtained in each study group [59].

The results of the continuous quantitative variables will be presented in measures of central tendency: mean, standard deviation and/or range.

- Intragroup analysis: Differences within groups will be analyzed using the one-way ANOVA or the Student's t-test when applicable.
- Inter-group analysis: For equal variances, Tukey's post-hoc test will be used, otherwise Dunnett's statistic will be used. Student's t-test will be used when applicable.

The level of difference to consider significance will be an alpha (α) of 0.05 or less. A 95% confidence interval (95% CI) will be considered for non-inferiority criteria [60].

The result of the nominal and ordinal qualitative variables will be presented in frequencies, proportions and percentages.

The statistical analysis to identify significant differences in the qualitative variables will be carried out by creating 2x2 contingency tables and will be carried out as follows:

- Intra-group difference: McNemar test. This is applied to 2×2 contingency tables with a dichotomous feature, with pairs of paired subjects, to determine if the marginal frequencies of row and column are equal (marginal homogeneity).
- Difference between groups: Pearson's Chi-square test (X^2) or Fisher's exact test at expected values less than 5.

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

For the reporting of AEs, 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 subjects.

The final report of the results will be shown in tables or graphs, as appropriate.

10.1.3 Procedure for handling missing data

Safety: The safety assessment will include in the analysis all those subjects (both eyes) who have been exposed at least once to the intervention, regardless of the visit in which they were eliminated from the study (ITT; *Intention To Treat* population).

Efficacy: Those subjects who meet a minimum adherence of 70% will be included in the statistical analysis to meet the objective of the study, taken from the weight of the IP. In cases where the container is not returned, or it has not preserved its physical integrity, adherence will be measured by means of the subject's diary.

10.1.4 Deviations from the statistical plan

According to the calculation of the sample size to meet the primary objective of the study, 141 evaluable subjects (47 subjects per dosage) are required. If this number is not met due to a loss of subjects greater than 20% contemplated in this protocol (loss of follow-up or withdrawal of ICF), in order to balance the treatment groups, the sponsor may replace these subjects.

The results obtained from the substituted subjects will continue to be used for safety analysis and will be part of the *Intention To Treat* population (ITT).

10.1.5 Subjects included in the analysis

Those subjects who meet a minimum adherence of 70% by weight and 70% per diary will be included in the statistical analysis to meet the primary endpoint of the study (*Per protocol* population, PP).

10.2 Sample size calculation

10.2.1 Number of subjects calculated

n= 141 evaluable subjects (OU).

47 subjects per group.

10.2.2 Justification of the sample size calculation

The sample size calculation was made based on the study by Pinto-Fraga et al. (2017), where they evaluated the safety and efficacy of an artificial tear of 0.2% hyaluronic acid (HA) versus SS (saline, 0.9% NaCl) in patients with mild dry eye (OSD) after one month of treatment. Subjects treated with HA had a decrease of -19.66% with respect to their initial mean value, while in subjects treated with SS there was a significant increase in the final OSDI score of 12.47%, $p<0.05$ compared to the initial [61].

For the calculation, a decrease in the OSDI score of 20% was considered for the formulation proposed in this protocol (Multi-dose Lagricel® Ofteno) with respect to its initial mean value in subjects with mild to moderate DED.

The sample size was calculated using the equation for two proportions [62], considering a power of 80% (β), a significance level of 0.05 (α) and a non-inferiority margin (δ) of 10%, based on the following working hypotheses:

$$H_0: P_A - P_B \leq \delta$$

$$H_1: P_A - P_B > \delta$$

Where, δ is the margin of non-inferiority and the ratio between the sample size of the two groups is:

$$k = \frac{n_A}{n_B}$$

The calculation to estimate the sample size and power was done using an online tool and following the equations [63]:

$$n_A = kn_B, n_B = \left(\frac{P_A(1-P_A)}{k} + P_B(1-P_B)\right)\left(\frac{z_{1-\alpha} + z_{B-\beta}}{P_A - P_B - \delta}\right)^2$$

$$1-\beta = \phi(z - z_{1-\alpha}) + \phi(-z - z_{1-\alpha}), z = \frac{P_A - P_B - \delta}{\sqrt{\frac{P_A(1-P_A)}{n_A} + \frac{P_B(1-P_B)}{n_B}}}$$

Where:

- (k = $\frac{n_A}{n_B}$) is the allocation ratio,
- (Φ) is the standard normal distribution function,
- (α) is the Type I error,
- (β) is the Type II error, meaning that (1 - \beta) is the power, and
- (δ) is the test margin.

According to the previous calculation, the result is 39 subjects, this calculation was increased by 20% considering possible losses (8 subjects). The total suggested sample size is 141 subjects (47 subjects per treatment dosage), who will contribute OU to the study.

11. Ethical considerations

11.1 Committee approval

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

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

The study will not be initiated in the research centers if there are no confidentiality agreements and economic proposal of each of the principal investigators, duly signed and without having previously obtained the favorable opinion and/or approval of the RECs, RC, and when applicable by the corresponding Biosafety Committee.

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

The study is considered to be research with a greater than minimum risk, in accordance with the Regulations of the General Health Law on Health Research, Title Two, Chapter I, Article 17, Section III, published in the Official Gazette on January 6, 1987[3].

11.2 Amendments to the protocol

The amendment procedure will be pertinent when there is a need to make any change to a document that is part of the research project or protocol, derived from variations in the methodological structure, substitution of the principal investigator or in the face of the identification of risks in the research subjects. The documents that can be amended will be: protocol, letter of informed consent, investigator's manual, documents for the patient, measurement scales and schedule of activities.

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

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

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

11.3 Early termination of the study

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

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

1. The presence of serious AEs in more than 10% of participants in a study group.
2. The regulatory authority (COFEPRIS) considered it for safety alerts.
3. The Sponsor determined it for its convenience or eventualities such as: financial

support, manufacturing errors, etc.

4. The identification of unexpected risks to the participants, which are significant or unacceptable.
5. Obtaining new relevant safety information.
6. Insufficient adherence to the requirements of the protocol.
7. The data obtained are not assessable or are not sufficiently complete.
8. The determination that the primary objective has been achieved.
9. The determination of futility.

In the event of suspension, the study may be resumed once the situations that led to the suspension have been corrected; as long as this justification is sufficient for the sponsor, RC, REC and regulatory authorities.

11.4 Informed consent

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

The ICF will be considered as a source document and will be filed as such. The site's principal investigator is responsible for ensuring that all new versions of the informed consent are submitted to the appropriate approvals (the same as the consent letter original report was submitted) and that the most current approved version is the one presented to the study subjects

11.4.1 Obtaining of the ICF

The ICF must be obtained before the subject undergoes any procedure indicated in the protocol. For this purpose, the letter of informed consent must be signed.

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

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

This information will be in a language understandable to the subject, it will be explained to the subject that he or she has the right to interrupt his or her participation in the study at any stage, without this

affecting the relationship with the researcher and/or his or her future assistance. Informed consent will be put to the consideration of the potential participant; He must have enough time to analyze each and every one of the aspects mentioned above and in case he has any doubts, it will be clarified by the person in charge of obtaining the informed consent.

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

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

The PI will also need to sign and date this consent.

The ICF must be signed in duplicate by all those involved, and two witnesses, one copy will be filed in the researcher's folder and the other will be given to the participant. The PI or delegated personnel must document the process of obtaining the Informed Consent by means of a detailed medical note, specifying the signed version, the date on which the document was signed and how the process was carried out.

11.5 Special considerations

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

11.6 Modifications to the ICF

Any changes to the ICF constitute an amendment to this document and must be submitted for approval to the RECs, and if applicable to the Competent Authorities.

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

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

11.7 Confidentiality

All documents and information provided to the research center by the sponsor are strictly confidential. PI expressly agrees that data about your professional and clinical experience, provided to the sponsor on paper and stored in electronic form, is solely for use related to your activities with the clinical trial sponsor, in accordance with Good Clinical Practice.
The investigator agrees that he and his team members will use the information only within the framework of this study, to carry out the protocol. This agreement is binding as long as the confidential information has not been disclosed to the public by the sponsor.

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

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

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

All eCRFs and communications related to study subjects will identify them only by the study subject identification number. The information collected in this study will be exchanged between the sponsor and the research center, and must be treated confidentially. The Health Authority, the REC, the RC, the sponsor, the monitors/auditors and third-party auditors will be the only bodies authorized to review the study documentation. If publications arise from this research project, in no case will they

contain information on the identification of the study subjects. If the results of the study are published, no personal information of the study subjects will be revealed.

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

11.8 Conflict of interest

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

11.9 Declaration of interests

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

11.10 Access to information

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

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

11.11 Ancillary and post-study care

Once the study is completed and AEs are closed according to section: 8, the sponsor will not extend care to the research subject.

12. Biosecurity aspects

NO BIOSECURITY IMPLICATIONS

This protocol, entitled: "Phase II clinical study, to evaluate the efficacy of the Multi-dose Lagricel® Ofteno ophthalmic solution in 3 different dosages for the management of mild to moderate dry eye", and number: SOPH037-1219/II HAS NO 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 research center's staff or research subjects or affects the environment. It is also declared that this project will not carry out cell, tissue or organ transplant procedures, or cell therapy, nor will laboratory, farm or wildlife animals be used.

13. Publication policy

13.1 Final Report

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

13.2 Communication of results

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

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

13.3 Publication of results

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

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

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

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

14. Financing and insurance

14.1 Compensation to study participants

Subjects who participate in the study will not receive financial compensation for their participation in the study. However, randomized subjects will receive financial support for travel expenses at each scheduled visit to which they attend punctually. Such support, as well as the amount. It will be specified in the informed consent letter.

14.2 Insurance for study participants

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

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

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16. Annexes

16.1 OSDI

Identification card
No. of Study: SOPH037-1219/II **Date:** / /
Subject's initials: _____ **Subject No.:** 037-_____-

Directions:

The OSDI test is a simple test created to establish a severity/classification of dry eye according to its symptoms. Answer the following questions by checking the box that best represents your answer:

Over the past week, Have you experienced any of the following alterations?	Frequency				
	In every moment	**In almost every moment**	**50% of the time**	**Almost in no moment**	**In no moment**
1. Light sensitivity	4	3	2	1	0
2. Gritty feeling in the eyes	4	3	2	1	0
3. Eye pain	4	3	2	1	0
4. Blurred vision	4	3	2	1	0
5. Poor vision	4	3	2	1	0
Subtotal of the answered cells					(A)

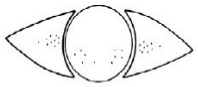
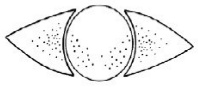
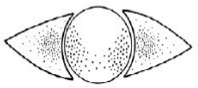
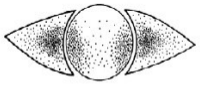
Over the past week, Have you had eye problems that have limited you to perform these actions?	Frequency					
	In every moment	**In almost every moment**	**50% of the time**	**Almost in no moment**	**In no moment**	**NA**
6. Read	4	3	2	1	0	NA
7. Driving at night	4	3	2	1	0	NA
8. Working with a computer or an ATM	4	3	2	1	0	NA
9. Watch TV	4	3	2	1	0	NA
Subtotal of the answered cells					(B)	

Over the past week, Have you felt discomfort in eyes with any of the following situations?	Frequency					
	In every moment	**In almost every moment**	**50% of the time**	**Almost in no moment**	**In no moment**	**NA**
10. Wind	4	3	2	1	0	NA
11. Places with low humidity (dry)	4	3	2	1	0	NA
12. Air-conditioned areas	4	3	2	1	0	NA
Subtotal of the answered cells					(C)	

Sum of A+B+C = (D)

Number of items answered, do not include responses as N (E)

16.2 Oxford Scale

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

16.3 Efron scale