Excelencia en oftálmicos

Protocol for the study

SOPH087-0120/IV

Title of the study: Phase IV non-inferiority clinical trial to compare the efficacy of Humylub Ofteno® PF ophthalmic solution against Hyabak® and Lagricel Ofteno® PF on the ocular surface of patients with mild to moderate dry eye syndrome.

This protocol has been developed in accordance with the principles of the Declaration of Helsinki and will be conducted in accordance with Good Clinical Practice and in compliance with ICH guidelines and applicable local legislation.

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

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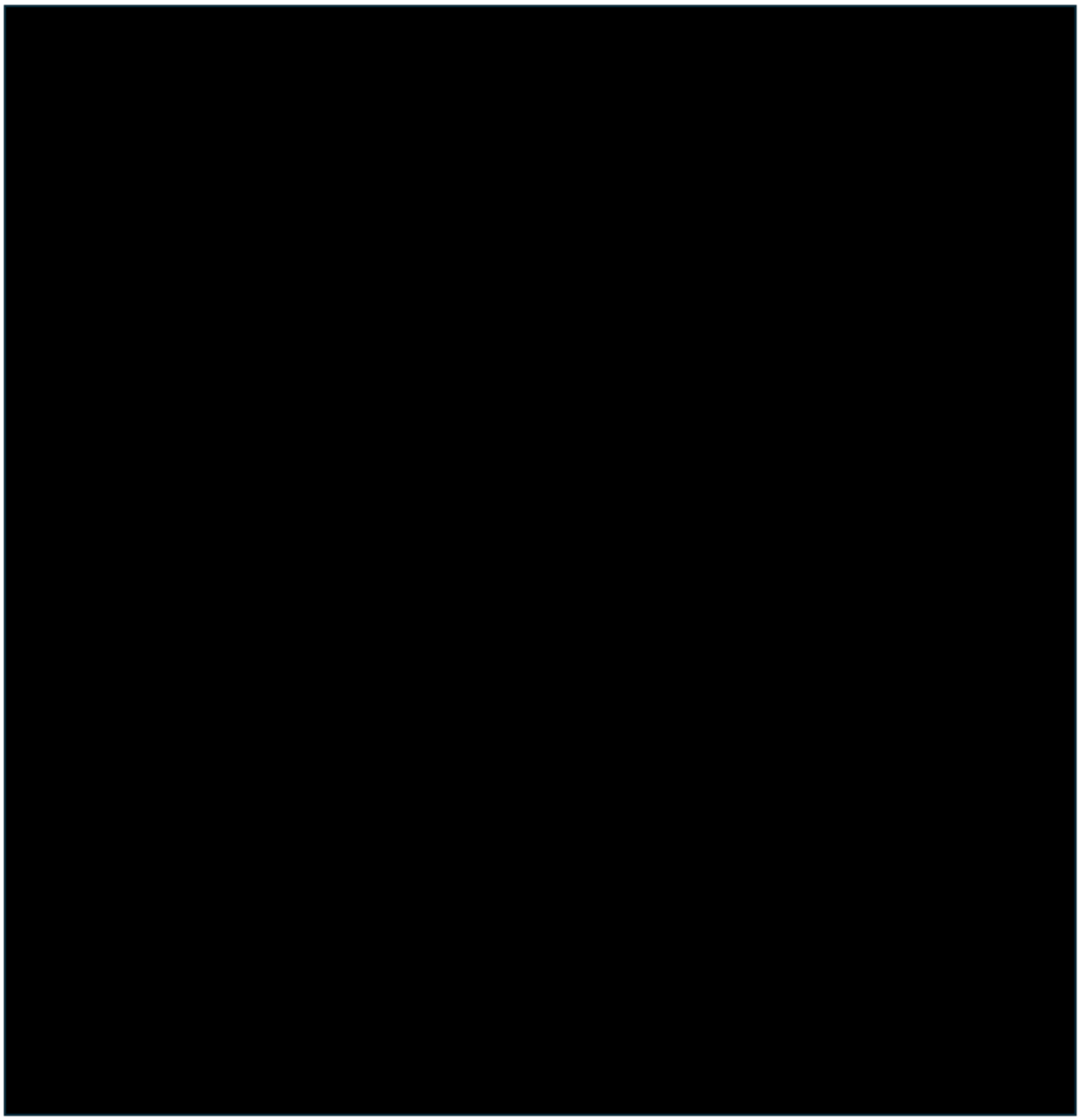
Responsible for the study

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

Signature Pages

From the sponsor



Investigator Agreement

I agree to conduct this clinical study according to 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 (GCP).

I agree to report all information or data in accordance with what is indicated in the protocol, particularly any AEs. 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:	
	Signature
Title:	
	Date
Name of the center:	
Geographic location (city/state/country)	

List of abbreviations

AH	Hyaluronic acid
ANOVA	Analysis of variance test
BPC	Good Clinical Practices
CIS	Research Ethics Committee
COFEPRIS	Federal Commission for the Protection against Sanitary Risks
IUD	Intrauterine device
EA	Adverse Event
FRCE	Electronic Case Report Form
FCI	Informed consent form
HS	Sodium Hyaluronate
ICH	International *Council for Harmonisation*
IP	Principal Investigator
ITT	Intent to Treat
MAVC	Better corrected visual acuity
OD	Right eye
WHO	World Health Organization
YOU	Left eye
SIDA	Ocular *surface disease index*
PEGs	Polyethylene glycol oligomers
PNA	Unanticipated problems
PIO	Intraocular pressure
PI	Research Products
QID	Four times a day
RNEC	National Clinical Trials Registry
SCE	Foreign body sensation
SOS	Dry eye syndrome
TFOS DEWS II	*Tear Film and Ocular Surface Society Dry Eye Workshop II*
TRL	Tear rupture time
TRLNI	Non-invasive tear rupture time

1. Summary

1.2 Synopsis

Title of the study: Phase IV non-inferiority clinical trial to compare the efficacy of Humylub Ofteno® PF ophthalmic solution against Hyabak® and Lagricel Ofteno® PF on the ocular surface of patients with mild to moderate dry eye syndrome	
Study number: SOPH087-0120/IV	**Data created:** 15-ene-20
Protocol version: 2.0	**Date of version:** 27-abr-20
Therapeutic indication: Ocular lubricant	**Use:** **Dry eye**
Estimated duration of the study (from the first visit of the first patient to the preparation of the final report): 12 months	**Clinical development phase: IV**

Objectives:

Objective principal:

- Demonstrate the non-inferiority of Humylub Ofteno® PF compared to Hyabak® and Lagricel Ofteno® PF in the efficacy of treating patients with dry eye, using the OSDI (ocular surface disease index) test score

Secondary objectives:

- Demonstrate the non-inferiority of Humylub Ofteno® PF compared to Hyabak® and Lagricel Ofteno® PF in the efficacy of treating patients with dry eye, through non-invasive measurement of tear breakup time (TBUT).
- Demonstrate the non-inferiority of Humylub Ofteno® PF compared to Hyabak® and Lagricel Ofteno® PF in the efficacy of treating patients with dry eye, through changes in corneal and conjunctival staining with fluorescein.
- Demonstrate the non-inferiority of Humylub Ofteno® PF compared to Hyabak® and Lagricel Ofteno® PF in the efficacy of treating patients with dry eye, based on changes in corneal and conjunctival staining with lysamine green.

- Demonstrate the non-inferiority of Humylub Ofteno® PF compared to Hyabak® and Lagricel Ofteno® PF in the efficacy of treating patients with dry eye, based on changes in conjunctival hyperemia.
- Demonstrate the non-inferiority of Humylub Ofteno® PF compared to Hyabak® and Lagricel Ofteno® PF in the safety of treating patients with dry eye, based on the incidence of chemosis.
- Demonstrate the non-inferiority of Humylub Ofteno® PF compared to Hyabak® and Lagricel Ofteno® PF in the safety of treatment of patients with dry eye, based on the incidence of adverse events (AEs).

Hypotesis:

H_0= The Humylub Ofteno® PF ophthalmic solution is not inferior in efficacy to its comparators after 30 days of treatment, showing a 35% decrease in the final OSDI test score from baseline in at least 30% of exposed patients.

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

H_1= The Humylub Ofteno® PF ophthalmic solution is inferior in efficacy to its comparators after 30 days of treatment, as it does not show a 35% decrease in the final OSDI test score from baseline in at least 30% of exposed patients.

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

Study design:

Phase IV non-inferiority, controlled, open-label, comparative, multicenter clinical trial.

Number of subjects (planned and analyzed):

Number of patients planned: 60 evaluable patients per arm.

Total sample = 180 evaluable patients.

Diagnosis and main inclusion criteria:

- Patients diagnosed with mild to moderate dry eye.

Selection criteria:

Inclusion criteria:

- Can voluntarily give their signed informed consent.
- Be able and willing to comply with scheduled visits, treatment plan, and other study procedures.
- Be of legal age.
- Women of childbearing potential must ensure the continuation (initiated ≥ 30 days prior to signing the informed consent form [ICF]) of the use of a hormonal contraceptive method or intrauterine device (IUD) during the study period.
- Present a diagnosis of mild to moderate dry eye, defined by an OSDI rating ≥ 13 and one of the following:
    - Corneal staining with more than 5 sites.

- Conjunctival staining with more than 9 sites.
- Tear film breaking time < 10 seconds.

Exclusion criteria:

- Women who are pregnant, breastfeeding, or planning to become pregnant within the study period.
- Have participated in another clinical research study ≤ 30 days prior to the screening visit.
- Have previously participated in this study.
- Have a CVAM of 20/200 or worse in one of the eyes.
- Present an ophthalmological diagnosis of:
  - Allergic, viral, or bacterial conjunctivitis.
  - Blefaritis anterior.
  - Parasitic infestations in any ocular structure or its appendages.
  - 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.
  - Disorders of the eyelids that cause palpebral malpositions, limit the proper closing or opening of the eyelids, or are the cause of epiphora.
- Have a management of your dry eye that requires the implementation of all the treatments in stage 2 of the recommendations on the treatment and staged management for dry eye disease of the TFOS DEWS II (Tear Film and Ocular Surface Societies II Dry Eye Workshop, Tear Film and Ocular Surface Society Dry Eye Workshop II).
- Have a history of drug addiction or drug dependence current or within the last two years prior to signing the FCI.
- Have a history of eye surgical procedure within the last 3 months prior to signing the FCI.
- Be a soft or hard contact lens wearer. You will be able to enter in case of suspending its use during the study, you must comply with 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 investigational products

Research Product (PI):

Investigational product, dosage and route of administration:

- Humylub Ofteno® PF. Sodium Hyaluronate 0.1% / Chondroitin Sulfate 0.18% Ophthalmic Solution. Laboratorios Sophia, S.A. de C.V., Zapopan, Jalisco, Mexico.
- Dosage: 1 drop 4 times a day (QID), with a minimum interval of 3 hours between applications.
- Route of administration: Ophthalmic

Reference products, dosage and route of administration:

Hyabak®. Sodium Hyaluronate 0.15% Ophthalmic Solution. *Laboratoires* Théa, Clermont-Ferrand, France.

- Dosage: 1 QID drop, with a minimum interval of 3 hours between applications.
- Route of administration: Ophthalmic.

Lagricel Ofteno® PF. Sodium Hyaluronate 0.4% Ophthalmic Solution. Laboratorios Sophia, S.A. de C.V., Zapopan, Jalisco, Mexico.

- Dosage: 1 QID drop, with a minimum interval of 3 hours between applications.
- Route of administration: Ophthalmic.

Duration of treatment:	Duration of the subject in the study:
30 days	Up to 41 days

Evaluation criteria:

Primary outcome variables

- OSDI score (ET: visits 1 and final).

Secondary outcome variables:

- Changes in the measurement of the TRLNI (ET: visits 1 and final).
- Changes of corneal and conjunctival stains with lysmine green (ET: visits 1 and final).
- Corneal and conjunctival staining changes with fluorescein (ET: visits 1 and final).
- Conjunctival hyperemia changes (ET: visits 1 and final).

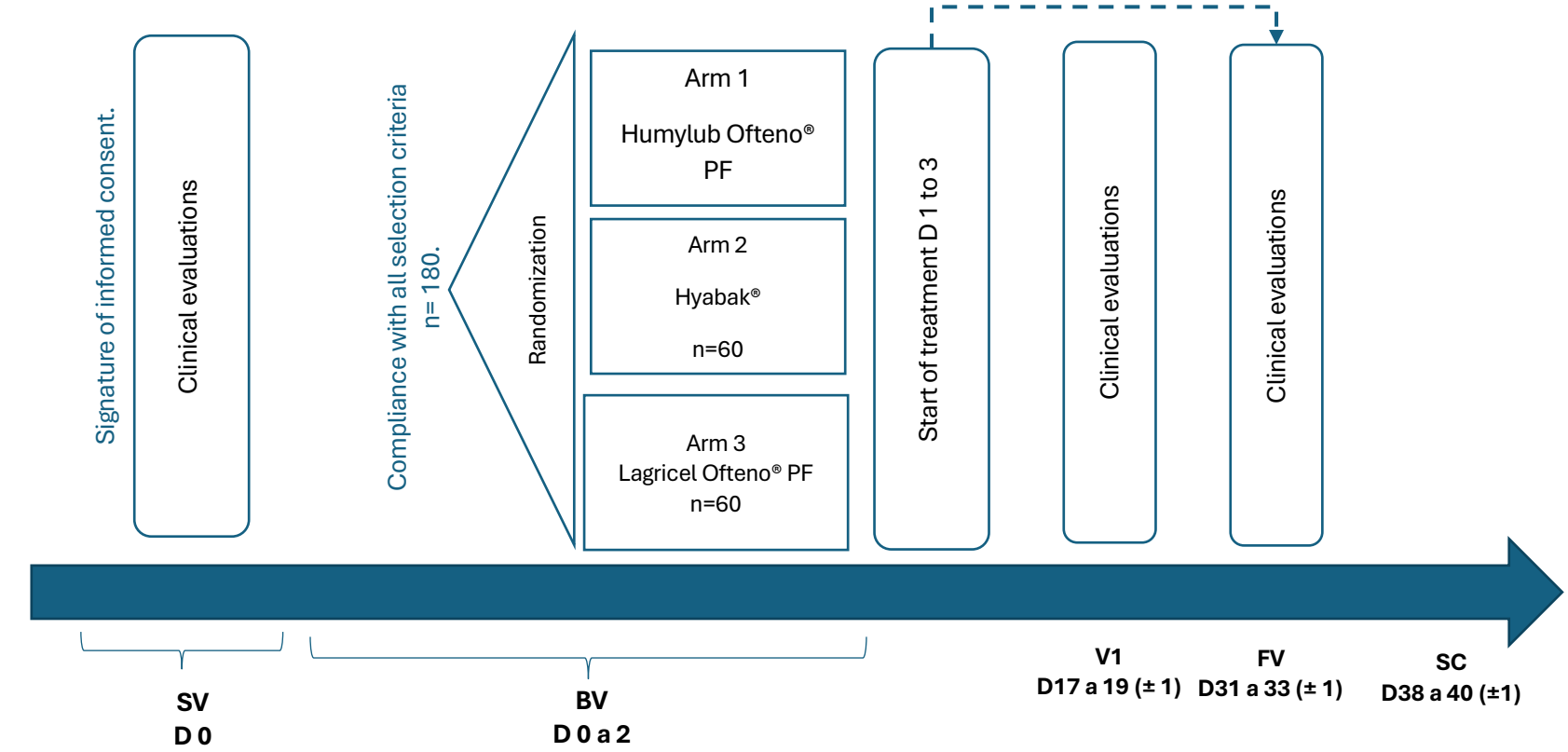
Safety variables:

- Incidence of unexpected AEs related to the research product. (ET: visits 1, final and evaluation)
- Changes in the MAVC (ET: visits 1 and final).
- Incidence of chemosis (ET: visits 1 and final).

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 the *X2 (Chi-square) or Fisher's exact test. A non-inferiority margin (*δ) of 10% will be used for the OSDI score based on the primary endpoint of the study, a $\alpha \leq 0.05$ will be considered significant.

1.2 Study diagram



SV = screening visit
BV = baseline visit
V1 = visit 1
FV = final visit
SC = safety call

Figure 2. Study diagram

1.3 Patient Timeline

The screening visit will correspond to an appointment that will be considered on day 0 for each patient. The baseline visit should be carried out 0 to 2 days after the scrutiny visit and can therefore be carried out on the same day as the latter.
Visit 1 will be carried out in relation to the start of treatment on day 17, with a window period of ±1 day.
The final visit will take place on day 31 of the study, in relation to the start of treatment, with a window period of ±1 day.
The safety call will be made 38 days after the start of treatment, with a window period of ±1 day.

Procedures	SV D 0	BV D 0 to 2	D 1 to 3	V1 D 17 to 1 (±1)	FV D 31 to 33 (±1)	SC D 38 to 40 (±1)
FCI Signature	X					
Medical history	X					
Concomitant Drug Evaluation	X			X	X	
Urine pregnancy test	X				X	
Vitals signs	X			X	X	
BCVA	X			X	X	
Ocular Surface Integrity (Stains and Evaluation of Hyperemia and Conjunctival Chemosis)	X			X	X	
Comprehensive ophthalmological evaluation	X			X	X	
OSDI	X			X	X	
Eligibility Criteria	X	X				
AE´s Assessment	X	X		X	X	X
TBT		X		X	X	
Investigational Product (PI) Randomization		X				
Delivery of the PI		X				
Delivery of patient material and filling instructions		X				
Start of intervention			X			
Adherence assessment				X	X	
Patient Journal Return / Evaluation					X	
Return of PI					X	

Table 2. Patient schedule

2. Introduction and Background

2.1 Theoretical framework

The stability of the tear film is the main characteristic of a healthy ocular surface, as it allows it to fulfill its main functions such as forming an adequate refractive surface and providing protection and moisture to the cornea. The loss of homeostasis of this layer of 2 – 5.5 µm causes dry eye syndrome (SOS). [1]

The tear film is composed of two layers, one lipid and one mucoaqueous. The lipid layer, thanks to its wax content and cholesterol esters, is generally attributed with preventing evaporation and tear rupture; however, this function is now considered to be fulfilled thanks to the interactions of all the compounds of the tear film, including lipids, mucins and salts. [1]

SOS is a multifactorial disease that affects millions of people around the world, with a prevalence ranging from 5 to 50%. It is a symptomatic condition characterized by a vicious circle of tear film instability and hyperosmolarity that causes inflammation of the ocular surface and neurosensory alterations. Being one of the main causes of ophthalmological consultation, it significantly impacts the lives of patients who suffer from it, as it is associated with: pain, limitation in the execution of activities of daily living, reduction of vitality and even depression. [2]

Economically, it also represents one of the most relevant diseases within ophthalmological diseases, with an estimated annual expenditure of 3.8 billion dollars related to SOS in the United States of America alone. [2]

Some of the risk factors associated with SOS are advanced age, female sex, use of contact lenses, systemic diseases such as Sjögren's Syndrome and Diabetes Mellitus, Asian race, use of screens, nutritional factors, refractive surgery, bone marrow transplant, meibomian gland dysfunction, and environmental factors. [2, 3]

According to its etiology and pathophysiology, SOS can be divided into two categories, which are not mutually exclusive, the aqueefficient type and the evaporative type. Epidemiology suggests a preponderance of the latter. [3, 4]

Regardless of the etiology and severity of SOS, the TFOS DEWS II recommends symptomatic evaluation of affected patients through a symptom questionnaire such as OSDI. See Annex 1 16.1 OSDI. This tool is reliable and has proven to be reliable both in detecting SOS and in classifying the degree of severity of the condition. [5, 6]

In addition, a complete ophthalmologic evaluation should be performed that includes the examination techniques necessary to evaluate the signs related to SOS. For the confirmation of this pathology, any of the following are considered evidence of alteration of tear homeostasis: reduction of TRLNI, hyperosmolarity or uptake of stains on the corneal, conjunctival or palpebral margin surface. [3]

The assessment of conventional tear rupture time (LRT) is performed invasively, by instilling fluorescein on the ocular surface. However, this method generates a destabilizing action of the tear film due to the increase in volume represented by the application of the stain, in addition to the production of reflex tears secondary to contact with said substance and exposure to intense light during the review in the slit lamp. [7] Because noninvasive methods of measuring TRL avoid this disruption of ocular surface balance, they are considered superior for the determination of this parameter. Currently, the use of equipment with computational algorithms that evaluate TRL has made it possible to ensure its objective and automated detection. [7] Among the options available today for the determination of TRLNI (non-invasive tear rupture time) is the Oculus Keratogaph 5M. It measures TRL by detecting localized tear film breaks using infrared waves. 22 rings are projected onto the corneal surface, with more than 1,000 measurement points per ring, while a real-time video record is taken. The evaluation yields two readings, the time it takes to observe the first tear film rupture, and the average time to show tear tear rupture in all monitored regions. [8]

In a study of 126 patients diagnosed with mild to moderate dry eye syndrome, Lee et al reported an average of 8.39 sec of 8.39 sec of LRT assessed by Keratograph 5M. [8] On the other hand, Bandlitz et al described an average of 12.9± 6.8 sec of TRL in apparently healthy patients. [9] Although variable results have been reported when comparing TRL values measured by conventional method with TRLNI, the TFOS DEWS II has established a TRLNI of 10 sec as the cut-off criterion for the diagnosis of SOS. This test is considered to have a sensitivity of 82 to 84% and a specificity of 76 to 94% for the diagnosis of this pathology. [10, 6, 11]

Once diagnosed, there are many therapeutic options for the treatment of SOS, with the main goal of its management being the restoration of homeostasis of the ocular surface and tear film. In general, the treatment of patients suffering from SOS begins with the indication of lubricants, which are accessible, low-risk and effective for the symptomatic treatment of the condition. [12]

Thanks to the rheological characteristics of eye lubricants, these products make it possible to relieve SOS-related discomfort. The properties of these ocular lubricants, including viscosity, lubricity, pH and osmolarity, have been described in other works. [13, 14, 12]

There are currently multiple formulations of tear substitutes, which can include the following components: an aqueous base, viscous agents, excipients, electrolytes, lipid supplements, buffers, and preservatives. However, the latter have important AEs. Some of the known alterations generated by conservators are cellular apoptosis of the corneal and conjunctival epithelium, damage to the corneal nerves, delayed corneal healing, interference with the stability of the tear film and loss of goblet cells. [12, 15]

Because the harmful effects of preservatives in ophthalmic formulations are widely documented, particularly in patients with chronic treatments, more and more attention has been directed to the development of dispensing devices that avoid the need to use such preservatives. [12] One of these is the Novelia® System, which allows patients to be offered a topical ophthalmic product presentation that reliably avoids contamination of the contents of the trophy without the need to use a preservative as part of the formulation.

2.2 Background on active ingredients

2.2.1 Background on Sodium Hyaluronate

2.2.1.1 Pharmacology of Sodium Hyaluronate

Sodium hyaluronate (HS), the sodium salt of hyaluronic acid (HA), is a naturally occurring glycosaminoglycan that has important biological functions in bacteria and higher animals, including humans. It is widely distributed in the cellular matrix of connective, epithelial, and neural tissue. It is present in cartilage, synovial fluid, in the skin and in the umbilical cord. In the eye it can be found in the vitreous humor and in the aqueous humor. [16, 12, 13]

It is synthesized on the inner side of the plasma membrane as a linear polymer, in contrast to other glycosaminoglycans which are synthesized by Golgi enzymes. The enzymes for the synthesis of HS are hyaluronate synthetases, which are part of the plasma membrane; and glycosyltransferases, which in coordination polymerize and translocate HS out of the cell into the extracellular matrix. [17] [18]

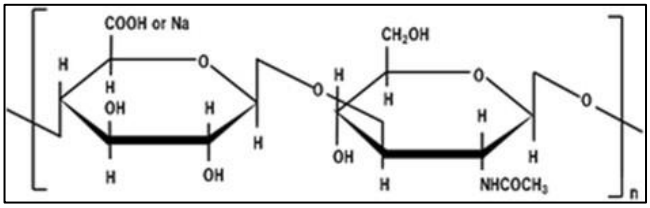


Figure 3. Chemical structure of HS

HS has several functions such as cell adhesion, migration, and proliferation. An important function is the ability to paralyze safe molecules in specific parts of the body by means of receptors (aggrecan, versican, neurocan, brevican, CD44 and RHAMM). [19] HS has been reported to be involved in several events during morphogenesis and differentiation. [17] [18]

It has the property of retaining water, and it has been determined that each HS molecule retains 1000 times its weight in water. This ability is compromised by factors such as sudden changes in temperature and pH. When the eye is open or the ocular surface is exposed, HS is more viscous, and coats the entire corneal surface, increasing tear film rupture time. If the eye is closed, HS spreads through the eye and eyelids, so it needs to be given again. [19]

Due to the properties HS has several physiological functions, one of the main ones is to act as a viscoelastic agent, being useful in the treatment of severe dry eye as it has viscous properties similar to mucin. It provides hydration and retention for long periods, and with its use good lubrication of the ocular surface is obtained. [19, 13]
Its beneficial effects on corneal healing have also been described. [13]

A hyaluronate receptor, CD44, has been shown to be expressed in corneal and conjunctival cells, and its activation promotes interaction with cytoskeletal proteins, suggesting a role for hyaluronate in cell adhesion and motility. It has also been proposed that hyaluronate may have a role in controlling

localized inflammation present in patients with SOS. It has been reported that CD44 expression is increased in patients with mild and moderate stages of the disease, and that, after a period of two months of application of HS, decreases the expression of this molecule. [13]

2.2.1.1.1 Pharmacokinetics in the eyeball

There is preclinical evidence on the pharmacokinetics of HS and HA after dermal, subdermal, intravenous, intraperitoneal, and intraocular administration. [20] However, no information is available on the pharmacokinetic profile of this molecule following topical ophthalmic application.

The corneal and conjunctival epithelium allows the passage of molecules through their cells by diffusion through the transcellular route and between their spaces through the paracellular route, for hydrophobic and hydrophilic substances, respectively. Molecular size is a limiting factor for the absorption of any substance through these structures. [21]

Hamalainen et al. (1997) characterized paracellular permeability in the cornea, conjunctiva, and sclera of albino rabbits New Zealand using polyethylene glycol oligomers (PEGs) such as HS and chondroitin sulfate. This study reported that both corneal and conjunctival permeability decreased proportionally with increasing molecular weight of the PEGs used. In addition, the concentration of PEGs in the subjects' anterior chamber did not present significant variations due to their small permeation fraction. The corneal permeability coefficient was 0.22 x 106 cm/sec for PEGs with a molecular weight of 942 g/mol.

The conjunctiva, both palpebral and bulbar, was 15 to 25 times more permeable than the cornea. This difference can be attributed to the fact that the conjunctival epithelium has wider paracellular pores (4.9 nm ± 2.5 and 3.0 nm ± 1.6, for palpebral and bulbar conjunctiva, respectively) compared to those of the cornea (2.0 nm ± 0.2).

Even considering the greater permeability of the conjunctiva compared to the cornea, the results of the tests on rabbit conjunctivae show that the neutral hydrophilic molecules that present penetration are those with molecular weights less than 1000 g/mol. [22]

Considering the above information, it can be concluded that HS and chondroitin sulfate (CS) molecules, which are classified as high molecular weight (800 Kg/mol), will not present intraocular penetration.

Evaluating its intraocular use, HA rotation was tested by injection into the anterior chamber of the eyes of New Zealand white rabbits. The concentration of the solution used was determined to be 10 mg/mL by radioassay. Two rabbits had 0.2 mL of aqueous humor extracted to be replaced by HA, and two more had 0.55 mL extracted and replaced. In addition, one rabbit was injected intramuscularly and another rabbit subcutaneously with 0.1 mL of the solution. Baseline blood samples were taken and 1, 2, 4, 8, and 24 hours, as well as daily for four days, and then every other day for 9 to 13 days after injections. Radiolabeling was detectable in the blood 2 to 3 hours after intraocular injection. It peaked at 2 days and then declined exponentially. The HA half-life at 0.2 mL injections was 14 hours and 8 hours for those with 0.55 mL injections. The hyaluronic acid injected subcutaneously had a half-life of 50 hours, and the intramuscular material of 30 hours.

In another experiment conducted on *cynomolgus monkeys*, HA was injected into the anterior chamber at a concentration of 10 mg/mL. After extracting 100 µL of aqueous humor, 50 µL of HA was injected into the eyes of 5 monkeys. The procedure was repeated using 75 µL in 3 monkeys and in 4 additional monkeys 50 µL plus pilocarpine addiction was injected, 10 µL at 4% instilled in the eye at hours 1, 8 and 24, after injection. The authors reported that radiolabeling was detected in blood 2 to 3 hours later, reaching the maximum in 2 to 3 days in animals that were not administered pilocarpine and after 1 day in the rest. This concentration decreased exponentially. The half-life for the 8 monkeys was 21 hours without the addition of pilocarpine, and 9.5 hours with the latter. [20]

2.2.1.1.2 Systemic pharmacokinetics

2.2.1.1.2.1 Preclinical studies

Balogh et al. (2008) determined the absorption, distribution, and excretion of high-molecular-weight hyaluronate radiolabeled with technetium following oral dosing to Wistar rats and Beagle dogs. All tissues examined showed incorporation of radioactivity from 15 min to 48 hours. A concentration of radioactivity in joints, vertebrae, and salivary glands was observed through a whole-body scan 4 hours after administration. An excretion of 86.7%- 95.6% of radioactivity in feces was reported. [23]

Another study examined the transfer of radioactivity to fetuses of pregnant rats that were injected intravenously with sodium hyaluronate. The fetuses' radioactivity increased up to 24 hours after injection and remained for up to 48 hours, before decreasing again after 72 hours. Radioactivity was distributed through the body of rats 1 to 4 hours after administration and decreased in whole-body tissues progressively. [20]

2.2.1.1.2.2 Clinical studies

After oral ingestion, absorption of a high molecular weight polysaccharide is difficult. It has been reported that HA with a molecular weight greater than 100 kg/mol is rarely absorbed by the intestinal epithelium. When ingested, enteric bacteria break down HA to its low molecular weight form, free polysaccharides that can travel to other systems such as joints. Approximately 90% of orally administered HA is metabolized and excreted through expiration and urine. [24]

In humans, after intravenous administration, HA has a plasma half-life of 2.5 to 5.5 minutes. From the circulation, it is mostly catabolized by the liver where it is degraded into acetate and lactate. The half-life of HA in cartilage has been reported to be 1 to 3 weeks. [25]

2.2.1.2 Efficacy of Sodium Hyaluronate

HA is a polymer with distinctive viscoelastic and hygroscopic properties that has demonstrated its therapeutic effect in patients with SOS. As an active ingredient, HA has shown an increase in the quality of life and satisfaction level (44.5% – 70.2%) of patients with SOS after 7 days of treatment. [26]

Saeed et al. studied the efficacy, safety, and tolerability of HA in 240 patients treated with a 0.4% HA ophthalmic solution. After 8 weeks of treatment, ocular signs and symptoms decreased compared to baseline, reporting a 5.3% difference in foreign body sensation (SCS) and pruritus. Similarly, Pito-Fraga

et al. reported a significant increase in patient satisfaction with mild SOS after being part of a one-month crossover study with 0.2% HA compared to 0.9% saline (26.8% vs 13.3%). [26]

Aragona et al. also described the efficacy of HS in patients with SOS who, after 3 months of treatment, showed improvement in the results of impression cytology. They concluded that long-term treatment with artificial tears containing HS reduces ocular surface damage in patients with SOS. [27]

Combinations of lubricants such as 0.1% HA with 0.5% carboxymethylcellulose have also been studied, finding this formulation effective in reducing SOS symptoms in patients recovering from cataract surgery, as evidenced by the OSDI questionnaire. [26]

HS, like other viscous materials, has been shown to have greater effects than other conventional aqueous artificial tears on decreased fluorescein uptake in patients with SOS.

These additional effects have been attributed to both the prolonged retention time and the physiological activity of HS. In a study published by Shimmura et al. comparing a 0.3% HS preservative-free solution with a non-viscous saline solution, it was observed that the HS formulation was more effective in reducing fluorescein staining in patients with SOS. [13]

2.2.1.3 Safety of Sodium Hyaluronate

2.2.1.3.1 Preclinical studies

Several studies of the safety of HA have been conducted. No toxicity has been observed in single-dose, repeated-dose, reproductive or developmental toxicity, mutagenicity, and antigenicity studies. [24]

In a study performed on a human conjunctival cell line by cytofluorometry with cold light microtitration to evaluate cellular tolerance to HS and look for a protective action *in vitro*. Various cellular functions such as cell viability, cell proliferation, and the production of reactive oxygen species were evaluated. 0.18% HA was used. The tests were carried out after 15 minutes of treatment and after 24 hours of recovery of the cells in the culture medium. Ocular viability was assessed by a neutral red fluorimetry test. Chromatin condensation was assessed by measuring total cellular DNA as a reference in the assessment of apoptosis. Each test was performed at least three times. No decrease in cell viability was observed with HA, nor any change in membrane integrity after 24 hours. [28]

For the evaluation of single-dose toxicity, >1200 mg/kg HA has been administered orally to mice. In addition, the toxicity of acute and chronic HA administered by different routes such as inhalation in Beagle dogs, implantation in the paravertebral muscles of rabbits, peritoneal administration in albino rabbits New Zealand and pleural administration in white rats has been evaluated. All the studies mentioned supported the safety of HA. [29]

It was reported that after vitreous humour supplanting in *rhesus* monkeys and owl monkeys, when assessing inflammation by leukocyte count in the aqueous humour, no significant differences were observed. Only two eyes of the owl monkeys and two *rhesus* monkeys showed slight vitreous turbidity, a reaction that disappeared between 72 and 96 hours for all subjects. These same authors repeated the procedure up to 6 times in owl monkeys, concluding that repeated HS implantation did not cause

increased turbidity and flare, as well as any other immune response. Even after a follow-up period of up to 9 years, in subjects who received 2 to 4 HS injections, it was concluded that all eyes were completely normal, with no alterations in the anterior segment, lens, vitreous, retina or choroid. [29]

With respect to dermal irritation, it was shown that HA does not cause alterations after a single dose in Japanese rabbits and Hartley guinea pigs. The ototoxicity of HA was also evaluated in pigmented Sprague-Dawley rats, concluding that the administration of this compound in the middle ear does not cause alterations to the auditory system. [29]

The reproductive and developmental toxicity of HS has been studied in preclinical studies *in vivo*. In a multigenerational study in Sprague-Dawley rats and in another conducted in Japanese white rabbits, HA solutions were administered subcutaneously at three different concentrations. Weights and feed intake, as well as the pathology of intraperitoneal organs and skeletal system after the sacrifice of the selected mothers and calves, were analyzed, without finding significant differences. There were also no differences between the intervened subjects and the control subjects when assessing gestation time, number of offspring, sex ratio, live embryos and fetuses, and viable fetuses. [29]

2.2.1.3.2 Clinical Trials

Aragona et al. (2002) reported that no AEs were present during a clinical study of efficacy and safety, after three months of treatment, in addition to good tolerability to the formulation. [27]

The immunogenicity of HS has been tested in humans, by allergy skin tests, without finding adverse reactions related to the compound. In addition, its safety has been reported when used in the treatment of vesicoureteral reflux and as an aid in tissue augmentation. [29]

2.2.2 Background on chondroitin sulfate

2.2.2.1 Pharmacology of chondroitin sulfate

Chondroitin sulfate is a proteoglycan, a class of high molecular weight glycoprotein that is highly glycosylated. It is an endogenous natural substance that is part of the physiological components of connective tissues, being one of the constituent elements of cartilage. It is part of the group of glycosaminoglycans, like sodium hyaluronate, it is a mucopolysaccharide found in the extracellular matrix of connective tissues, including the vitreous, cornea and aqueous humor. [25]

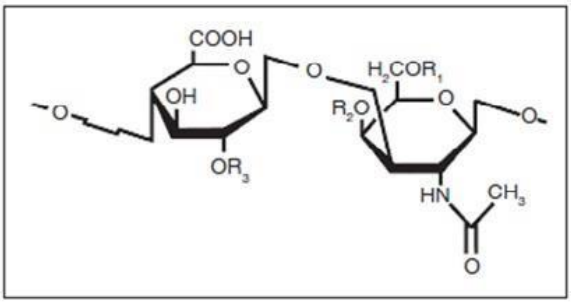


Figure 4. Chemical structure of CS

2.2.2.1.1 Pharmacokinetics in the eyeball

As for HS, there is currently no pharmacokinetic information following topical application of CS. However, due to its characteristics, particularly its high molecular weight, absorption by this route is not expected. To read more about this property and its interaction with the ocular surface epithelium, see section 2.2.1.1.1 Pharmacokinetics in the eyeball.

2.2.2.1.2 Systemic pharmacokinetics

CS, due to its high molecular weight, is partially absorbed in the intestine after being degraded to its polymeric form. The products of intestinal degradation are simple sugars, amino sugars and inorganic sulfate. Cueto-Galán et al. describe that the bioavailability of CS ranges from 15% to 24% of the orally administered dose. Of the absorbed fraction of CS, 10% is in the form of CS and 90% in the form of depolymerized derivatives of lower molecular weight, suggesting a first-pass effect. After oral administration of CS, the maximum concentration of CS in the blood is reached in about four hours.

In the case of intravenous administration, the plasma half-life of CS has been described as between 3-15 minutes, suggesting that the systemic effects of orally administered CS could be mediated by the intestinal immune system, including the gastrointestinal lymphoid system. The half-life of total CS is 15 hours, reaching a stationary period in 3-4 days. [25]

2.2.2.2 Efficacy of chondroitin sulfate

In 2013, Llamas-Moreno et al. published a clinical study sponsored by Laboratorios Sophia, S.A. de C.V. in which the efficacy and safety of a preservative-free ophthalmic solution containing chondroitin sulfate in combination with xanthan gum was evaluated, comparing it with a combination of polyethylene glycol/propylene glycol/hydroxypropyl guar. After two months of treatment in patients with mild to moderate SOS, it was reported that in both groups the TRL was similar, however, the OSDI test score showed a greater decrease in the chondroitin sulfate 0.09%/xanthan gum 0.1% group. [30]

The efficacy of this same combination was also studied in a phase III study published by Pérez-Balbuena, et al (2016). This was another comparative study in which it was compared with a solution of polyethylene glycol 400 0.4 % / propylene glycol 0.3 %. The variables studied in the 148 included patients with mild to moderate SOS were the TRL, OSDI test score, and Schirmer test. The results showed that both groups had similar clinical efficacy after two months of treatment. [31]

Limberg et al. (1987) published a clinical study in which 20 patients with keratoconjunctivitis sicca were treated with a viscoelastic formulation of chondroitin sulfate alone, another in combination with sodium hyaluronate, a third of sodium hyaluronate alone, and finally a polyvinyl alcohol solution. It was observed that treated patients showed marked improvement in terms of reduced pruritus, burning, and ECS severity. A reduction in corneal uptake and formation of mucosal filaments was also observed. It was reported that those patients who presented initial low results in the Schirmer test preferred the solution containing CS. [32]

2.2.2.3 Safety of Chondroitin Sulfate

In both studies previously mentioned in section 2.2.2.2 Efficacy of chondroitin sulfate published by Llamas-Moreno et al. (2013) and Pérez-Balbuena et al. (2016), the safety of the preservative-free ophthalmic solution composed of chondroitin sulfate 0.09%/xanthan gum.0.1% was analyzed by the incidence of ocular and systemic AEs during a period of 2 months of treatment. During this period, no AEs related to the intervention were presented in any of these studies, concluding that this formulation is safe. [30, 31]

2.3 Summary of pharmaceutical development of Humylub Ofteno® PF

Humylub Ofteno® PF has been developed at Laboratorios Sophia, S.A. de C.V. and has the physicochemical characterization and the protocol of accelerated and long-term stability.

In 2014, a preclinical safety and toxicity study was carried out in which Humylub Ofteno® PF was evaluated. This preservative-free formulation was compared with Humylub Ofteno®, after QID was applied for one month to the surface of both eyes of albino New Zealand rabbits. The primary variables evaluated were conjunctival hyperemia, corneal surface status, and histopathology toxicity data. Secondary endpoints included conjunctival secretion and other anterior segment alterations. During the execution of the study, only one subject with moderate hyperemia was presented, however, this finding was not statistically significant. In the same way, only one case of fluorescein staining was presented in the control group, without this being statistically relevant. The rest of the variables, including revision of the posterior pole, intraocular pressure, and staining with lysmine green, did not show alterations. Due to the results obtained, it was concluded that Humylub Ofteno® PF was safe, as was its comparator. [33]

Subsequently, a Phase I clinical study was conducted in which the safety and tolerability of Humylub Ofteno® PF was demonstrated compared to Xyel Ofteno® and Systane Ultra® products, on the ocular surface of ophthalmologically and clinically healthy subjects. As variables to evaluate tolerability, burning, ECS, pruritus, and ocular comfort index were analyzed. For the safety study, goblet cell density, presence of AEs, intraocular pressure, visual capacity, laboratory tests, epithelial defects in the cornea and conjunctiva, conjunctival hyperemia, and chemosis were considered. No statistically significant differences were observed between the density of initial goblet cells between the three treatments, nor in the final treatments. A total of 28 AEs were reported in 46.7% of the subjects in the total sample (14/30), of which 100% were classified as mild. For lysmine green staining, a statistically significant difference was observed at visit 2, while for fluorescein staining, statistically significant differences were observed at visits 1, 2, and final at the expense of a higher number of grade 0 cases in the comparator group. For conjunctival hyperemia, statistically significant differences were only observed at visit 1 where the group treated with
Systane Ultra® showed a higher frequency of normal values compared to Humylub Ofteno® PF and Xyel Ofteno®. No statistically significant differences were observed between the density of initial goblet cells between the three treatments, nor in the final treatments. In the variables evaluated, both safety and tolerability, Humylub Ofteno® PF showed a safety profile like that of the comparators, so it was concluded that it is a safe and tolerable formulation. [34]

Finally, in 2019, a multicenter phase IV study was carried out, in which the efficacy and safety of Humylub Ofteno® PF was evaluated compared to Systane® Ultra and Systane® Ultra free of preservatives. In this study, with a duration of 90 days, 326 patients were included. In efficacy variables, such as TRL, Schirmer's test, OSDI, and goblet cell density, all three treatment groups were effective, showing statistically significant improvement between their baseline and final visits. However, no group was consistently and statistically significantly superior to the other. For the variables defined as primary safety outcomes, such as presence of AEs, TRL, visual acuity and ability, intraocular pressure (IOP), and surface stains, no statistically significant differences were observed between groups in any of the measurement times. Finally, for the variables marked as tolerability (burning, SCE, tearing, and conjunctival hyperemia) there were no differences between groups; however, there was improvement in relation to the baseline visit. Based on the results presented above, it was concluded that Humylub Ofteno® PF is a safe and effective lubricant in the treatment of mild to moderate dry eye. [35]

2.4 Background on the investigation

2.4.1 From the research question

SOS is a multifactorial pathology whose treatment can include multiple topical medications, oral medications, and even surgical procedures. However, ocular lubricants represent the most widely used therapeutic tool by patients with any degree of disease severity. Despite this, there are few comparative studies that allow us to determine the superiority of any lubricant over the rest. [12] However, the properties of each active ingredient provide them with specific characteristics that are exploited to reduce symptoms and signs associated with SOS. There are many commercially available lubricant formulations, so this study aims to test the non-inferiority in efficacy and safety of Humylub Ofteno® PF compared to two other known products, Hyabak® and Lagricel Ofteno® PF.

2.4.2 From the investigational product development phase

Humylub Ofteno® PF is a product that is available for purchase without a prescription in Mexico and Latin America. It has a research and development profile that includes a preclinical *in vivo safety* and toxicity study, as well as a phase I clinical study and a previous phase IV study in which it was compared with Humylub Ofteno® with preservative. See point 2.3 Summary of the pharmaceutical development of Humylub Ofteno® PF.

2.5 Risk-benefit assessment

2.5.1 Known potential risks

Due to the characteristics of the active ingredients of Humylub Ofteno® PF, particularly its high molecular weight and the fact that it is composed of endogenous substances widely distributed in healthy organisms, the occurrence of serious adverse effects related to the investigational product is not expected. The safety profile of both assets, both individually and in combination, has been described in other studies. See points 2.2.1.3 Safety of sodium hyaluronate, 2.2.2.3 Safety of chondroitin sulfate, and 2.3 Summary of pharmaceutical development of Humylub Ofteno® PF.

As with the rest of the topical ophthalmic application formulations, the possibility of mild and transient burning, SCE and blurred vision is anticipated, with a post-instillation duration of no more than one minute. The procedures involved in the execution of this study, and the medications used for them, are also safe and have been described as part of the complete ophthalmological examination.

2.5.2 Known potential benefits

The use of ocular lubricants has been shown to decrease SOS-related signs and symptoms such as burning, SCE, corneal fluorescein uptake, etc. See points 2.2.1.2 Efficacy of sodium hyaluronate and 2.2.2.2 Efficacy of chondroitin sulfate. Patients participating in this study will be exposed to the potential benefit of presenting a significant clinical improvement in the clinical picture related to SOS, with the consequent improvement in their quality of life.

2.6 Problem statement

SOS is a disease that affects millions of people around the world. These patients present a disruption of the homeostasis of their tear film and may suffer multiple signs and symptoms associated with the inadequate protection of their ocular surface due to an insufficient tear in quantity or quality. Although there are multiple therapeutic options available, it is a pathology that continues to be studied due to its great social and economic impact. In addition to seeking therapeutic measures that eliminate the specific etiology of this syndrome, a priority of treatment is to eliminate the symptoms that cause a significant decrease in quality of life. This can be achieved thanks to the use of eye lubricants, whose rheological properties largely determine their effectiveness and safety. It is necessary to continue evaluating the available lubricants to assess their clinical effect and confirm their efficacy and safety in the population exposed to them.

2.7 Justification

The great impact that the diagnosis of SOS represents, both socially, economically and scientifically, has been established, without a doubt; but above all for the quality of life of patients who suffer from it. Epidemiological evidence describes that in some regions the incidence of this disease can reach up to more than 50% of the population. There are currently multiple therapeutic options for the management of SOS, among which ocular lubricants occupy a main place due to their effectiveness in reducing the symptoms associated with the condition and as an aid in the restoration of tear film homeostasis. In the search for the best formulation to manage this type of patient, the rheological properties of substances such as glycosaminoglycans, a family to which both active ingredients of Humylub Ofteno® PF belong, have been used. By combining HS and CS, it is possible to offer patients with SOS symptomatic relief, as well as improvement in signs and alterations related to the vicious circle that characterizes SOS.

Humylub Ofteno® PF is a product that is already marketed, and whose safety profile has already been previously studied; however, it was decided to carry out this study to compare its efficacy with two formulations containing sodium hyaluronate at different concentrations. The intention of this study is to confirm that patients with mild to moderate dry eye find an equal or greater decrease in their

symptoms with the use of Humylub Ofteno® PF, compared to reference products, and to continue in this way to expand the information available on this ophthalmic solution and strengthen its efficacy and safety profile.

3. Objectives and hypotheses

3.1 Primary objective

To demonstrate the non-inferiority of Humylub Ofteno® PF compared to Hyabak® and Lagricel Ofteno® PF in the efficacy of the treatment of patients with dry eye, by means of the OSDI (Ocular Surface Disease Index) test score.

3.2 Secondary objectives

- To demonstrate the non-inferiority of Humylub Ofteno® PF compared to Hyabak® and Lagricel Ofteno® PF in the efficacy of the treatment of patients with dry eye, by means of the measurement of the LRLR.
- To demonstrate the non-inferiority of Humylub Ofteno® PF compared to Hyabak® and Lagricel Ofteno® PF in the efficacy of the treatment of patients with dry eye, by means of changes in corneal and conjunctival stains with fluorescein.
- To demonstrate the non-inferiority of Humylub Ofteno® PF compared to Hyabak® and Lagricel Ofteno® PF in the efficacy of the treatment of patients with dry eye, by changing corneal and conjunctival stains with lysmine green.
- To demonstrate the non-inferiority of Humylub Ofteno® PF compared to Hyabak® and Lagricel Ofteno® PF in the efficacy of the treatment of patients with dry eye, through changes in conjunctival hyperemia.
- To demonstrate the non-inferiority of Humylub Ofteno® PF compared to Hyabak® and Lagricel Ofteno® PF in the safety of the treatment of patients with dry eye, through the incidence of chemosis.
- To demonstrate the non-inferiority of Humylub Ofteno® PF compared to Hyabak® and Lagricel Ofteno® PF in the safety of the treatment of patients with dry eye, through the incidence of AEs.
- To demonstrate the non-inferiority of Humylub Ofteno® PF compared to Hyabak® and Lagricel Ofteno® PF in the safety of the treatment of patients with dry eye, by means of changes in CVAM.

3.3 Hypothesis

H_0= The Humylub Ofteno® PF ophthalmic solution is not inferior in its efficacy compared to its comparators, after 30 days of treatment, presenting a 35% decrease in the final score of the OSDI test compared to its initial value in at least 30% of exposed patients.

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

H_1= The Humylub Ofteno® PF ophthalmic solution is inferior in its efficacy compared to its comparators, after 30 days of treatment, as it does not present a 35% decrease in the final score of the OSDI test compared to its initial value in at least 30% of exposed patients.

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

4. Study design

4.1 Design Overview

Phase IV non-inferiority, controlled, open-label, comparative, multicenter clinical study.

4.2 Rationale for the study design

Humylub Ofteno® PF is a product that is currently available for consumption not only in Mexico, but in several Latin American countries such as: Bolivia, Chile, Colombia, Costa Rica, Costa Rica, Colombia, Costa Rica, Colombia Dominican Republic, Ecuador, Guatemala, Haiti, Honduras, Nicaragua, Panama and El Salvador.

Both the efficacy and safety of its compounds, HS and CS, have been proven for use as a treatment for SOS, reducing both the signs and symptoms associated with this pathology. There are several ophthalmic solutions on the market that contain these compounds, including the comparators chosen for this study, Hyabak® and Lagricel® PF. The latter are composed of sodium hyaluronate, at different concentrations, and are also used for the treatment of SOS. One of the main purposes of SOS treatment is to reduce the symptoms described by patients such as burning, SCE, blurred vision, etc. The objective evaluation of these is carried out through internationally approved and accepted questionnaires, which are described in reference documents such as the TFOS DEWS II. One of these tools is the OSDI, the results of which will be considered in this study as the primary outcome variable. Like most efficacy studies for lubricating eye solutions, assessing improvement in patient-reported symptoms compared to reference products is the primary objective of this study. [36]

Due to the differences in the presentation of Humylub Ofteno® PF with one of its comparators, Hyabak®, and thanks to the characteristics of its bottles, this will be an open-label study.

Finally, considering the evaluation of the secondary variable of TRL efficacy by means of a non-invasive method, and since the equipment required to carry out this assessment is not so widely disseminated in ophthalmological offices and clinics in general, multiple research centers will be in charge of executing 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 12 months.

The planned recruitment period is 7 months. Considering that the proposed sample is 180 patients, the total average recruitment rate during the study should be no less than 0.86 patients per day. Competitive recruitment will be carried out among authorized research centers.

The approximate duration of each patient in the study is up to 41 days.

5. Study population

5.1 Eligibility Criteria

5.1.1 Inclusion criteria

- Can voluntarily give their signed informed consent.
- Be able and willing to comply with scheduled visits, treatment plan, and other study procedures.
- Be of legal age.
- Women of childbearing potential must ensure continued use (initiated ≥ 30 days prior to signing the CRF) of hormonal contraceptive method or IUD during the study period.
- Present a diagnosis of mild to moderate dry eye, defined by an OSDI rating ≥ 13 and one of the following:

  o Corneal staining with more than 5 sites. o Conjunctival staining with more than 9 sites. o Tear film breakdown time < 10 seconds.

5.1.2 Exclusion Criteria

- Women who are pregnant, breastfeeding, or planning to become pregnant within the study period.
- Have participated in another clinical research study ≤ 30 days prior to the screening visit.
- Have previously participated in this study.
- Have a CVAM of 20/200 or worse in one of the eyes.
- Present an ophthalmological diagnosis of:
  - Allergic, viral, or bacterial conjunctivitis.
  - Anterior blepharitis. o Parasitic infestations in any ocular structure or its appendages.
  - Unresolved eye trauma. o Healing diseases of the ocular surface. o Corneal or conjunctival ulcers.
  - Filamentous keratitis. o Neurotrophic keratitis. o Bullous keratopathy. o 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.
  - Disorders of the eyelids that cause palpebral malpositions, limit the proper closing or opening of the eyelids, or are the cause of epiphora.
- Have a management of your dry eye that requires the implementation of all stage 2 treatments of the TFO DEWS II treatment and stage management recommendations for dry eye disease.
- Have a history of drug addiction or drug dependence current or within the last two years prior to signing the FCI.
- Have a history of eye surgical procedure within the last 3 months prior to signing the FCI.
- Be a soft or hard contact lens wearer. You will be able to enter in case of suspending its use during the study, you must comply with 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 Patient Removal and Replacement Criteria

- Withdrawal of the letter from the FCI.
- Presentation of serious AEs related or not to the investigational product, which at the discretion of the PI and/or sponsor could affect the patient's ability to continue with study procedures safely.
- Non-tolerability or hypersensitivity to any of the compounds used during the tests (fluorescein, lysmine green, tetracaine).
- Non-tolerability or hypersensitivity to any of the investigational drugs.

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 Counting failures

A screening failure is defined as those participants who agree to participate in the study, giving their consent, but who are not assigned to 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 of Reporting Trials*) guidelines for the publication of results or to respond to possible questions from regulatory authorities.

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

5.5 Recruitment and Retention Strategies

This is a phase IV study, which is planned to be conducted at several research centers. The selected centers will be responsible for the recruitment of patients.

The minimum expected recruitment rate is 0.93 patients per business day. Because this study will be carried out in patients with mild to moderate SOS, a very common condition in the usual ophthalmological consultation, the participation of at least three research centers will be required.

The duration of the patient's participation in the study is approximately 41 days, during which time they will only have to attend three visits after the screening, however, the screening and baseline appointments can be made on the same day, so no retention problems are anticipated. However, patients will be entitled to travel support for transportation and to comply with their visits. Other strategies to improve patient retention include, but are not limited to:

- 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 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 patient does not last longer than necessary in his visit.
- Minimize patient wait times.

All materials to be delivered to the patient 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 patients who were randomized, who at some points were active patients in the study, but their final evaluation could not be completed.

If the participating patient does not attend their appointment, the research center must make a call to find out the reason and will attempt to make a new appointment within the established window period or an unscheduled appointment. In case 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 follow-up loss of <20% is considered not to be a problem for the validity of the results obtained based on the sample size calculations. See point 10.2 Calculation of the sample size.

5.7 Patient Identification

Study patients will be identified by a number and their initials.

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

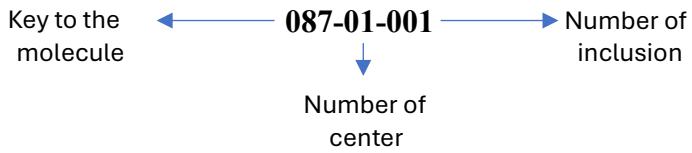
Example:

To. Arieh Daniel Carrizalez Market	B. Juan De la Torre Orozco
to. Initials: AMC	b. Initials: JDO

At the counting visit, the participant number will be assigned consecutively, using 3 consecutive digits. Once the patient 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 centre number.
- three digits of the number following their inclusion assigned in the research centre.

Example:



6. Investigational Product

6.1 Managed Products

6.1.1 Investigational Product

- Generic name: Sodium hyaluronate / Chondroitin sulfate.
- Distinctive name: Humylub Ofteno® PF.
- Active ingredients: Sodium hyaluronate 0.1% / Chondroitin sulfate 0.18%.
- Pharmaceutical form: Ophthalmic solution.
- Presentation: multi-dose dropper bottle, 10 mL.
- Prepared by: Laboratorios Sophia, S.A. de C.V.
- Solution description: Transparent solution, free of visible particles.
- Packaging description: White bottle made of low-density polyethylene with system Novelia® filled to 10 mL.

6.1.2 Reference product

- Generic name: 0.15% sodium hyaluronate.
- Distinctive name: Hyabak®.
- Active ingredients: Sodium hyaluronate 0.15%.
- Pharmaceutical form: Ophthalmic solution.
- Presentation: multi-dose dropper bottle, 10 mL.
- Prepared by: *Laboratoires* Théa, Clermont-Ferrand, France.
- Solution description: Transparent solution, free of visible particles.
- Packaging description: Abak® multidose bottle of 10ml.
- Generic name: Sodium hyaluronate
- Distinctive name: Lagricel Ofteno® PF.
- Active ingredients: Sodium hyaluronate 0.4%
- Pharmaceutical form: Ophthalmic solution.
- Presentation: multi-dose dropper bottle, 10 mL.
- Prepared by: Laboratorios Sophia, S.A. de C.V.
- Solution description: Transparent solution, free of visible particles.

- Packaging description: White bottle made of low-density polyethylene with Novelia system filled to 10 mL.

6.1.2.1 Justification of the reference product

Hyabak® is a preservative-free lubricant manufactured by Théa Laboratories, which is indicated for the treatment of SOS. [37] It is composed of 0.15% HS and is packaged in a multi-dose bottle. It is currently a direct commercial competitor of Humylub Ofteno® PF and Lagricel Ofteno® PF.

As it is composed of HS, it shares with the products of Laboratorios Sophia, S.A. de C.V. already mentioned, some of the benefits that it can offer to patients diagnosed with SOS. However, it lacks the association with CS as in the case of Humylub Ofteno® PF, and the properties attributed to a higher concentration of HS, as in Lagricel Ofteno® PF.

On the other hand, Lagricel Ofteno® PF is an ocular lubricant also manufactured by Laboratorios Sophia, S.A. de C.V. It has a broad safety profile from its development phase to the pharmacovigilance to which it has been subjected since its international commercialization, both in its single-dose presentation and in its multi-dose version, both free of preservatives.

6.1.3 Dosage of investigational product

One QID drop, with a minimum interval of 3 hours between applications, for 30 days.

6.1.3.1 Dose justification

The dosage of treatment for SOS based on ocular lubricants will depend on the severity of the clinical picture. In the case of this study, as these are patients diagnosed with mild to moderate SOS, a dose of 4 lubricating drops per day will be used in accordance with the dosage stipulated in the prescribing information of Humylub Ofteno® PF and Lagricel Ofteno® PF. In both cases, it is indicated to apply every 4 – 6 hours. On the other hand, the Hyabak® insert refers to a dosage of 1 drop in each eye as often as necessary. [37]

The requirement to wait at least three hours between each application is added to distribute the dosage throughout the day, ensuring the protection of the ocular surface during waking hours.

6.2 Storage and handling of research products at the study site

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

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

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

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

The storage temperature should be less than 25°C. This limit is stipulated as described in the Hyabak® comparator product information, since Humylub Ofteno® PF and
Lagricel Ofteno® PF require to be kept at a temperature below 30°C. [37]

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 PIs. 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 (PI) 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 PI in the event of a lack of undocumented material at the conclusion of the study.

6.3 Concomitant treatments and medications not approved during the study

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

Allowed medications:

- Ophthalmic:
    - Tetracaine 0.5%
    - Tropicamide 0.8% /Phenylephrine 5%

6.4 Procedure for monitoring and measuring adherence

For more than four decades, there has been a lot of research on the appropriate way to measure and quantify medication adherence, but none has reached a consensus to stand as the gold standard, both in cross-sectional and longitudinal studies.
[38] [39] [40] [41] [42] [43] [44] [45]

There are different procedures to measure adherence to pharmacological interventions. The most common procedure includes self-reports, which include patient interviews, questionnaires and self-monitoring diaries. Its strengths are speed, flexibility, low cost and ease of implementation; They have a high degree of specificity for non-adherence, however, the sensitivity and reliability for adherence is low. [45] [46]

The biochemical measurement of the drug, or its metabolite, is one of the methods that best confirms the use of the drug. However, in addition to raising costs and being impractical, it is of little use in the context of ophthalmic applications, since concentrations at the peripheral level could be undetectable, particularly in this study since ocular lubricants whose absorption is not expected will be evaluated. [45]

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) and
2. It depends on the patient bringing back the medication. [45] [46]

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 more accurately capture adherence levels. [44] There is no standardized parameter to define adequate adherence, it must be defined and delineated by the objectives of the research. [45]

Considering all the antecedents presented above, for this study adherence will be measured by the weight of the bottle and the record of applications of the patient's diary. A patient will be considered to comply with the required adherence if they have a positive record of applications in the patient's diary of at least 80%; as well as at least 65% of the decrease in the expected weight of the bottle used.

The evaluation of adhesion by means of the weight of the bottle will be carried out as follows, considering 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:

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

Where:

Ad = adherence
Pi = Initial weight
Pf = final weight
PT = Total treatment weight

$$P_T = (P_g)G$$

Where:

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

G = number of applications indicated for the intervention

The adherence calculation according to the patient's diary will be evaluated as follows:

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

Where:

Ad = Adherence

Ar = Registered Applications

Ai = Applications indicated for the intervention

The research center will designate a person in charge of monitoring adherence through the diary, during the visits. The measurement of grip by weight will be the responsibility of the sponsor.

In cases where the container is not returned, or it has not preserved its physical integrity, adherence will only be measured through the patient's diary.

6.5 Strategies to improve adherence

- The PI will make the patient aware of the importance, to achieve the objectives of the study, of the correct application of the PI.
- Direct questioning by the PI about the application of the PI.
- Delivery of a printed calendar specifying the date of the visit and its activities.
- Training in the completion and review of the Patient Diary.
- If deemed necessary, text messages may be sent as reminders. The content of these messages must be previously approved by the Ethics and Research Committee (CEI).

7. Study methods and procedures

7.1 From the research centre

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

The research centers will be responsible for forming a multidisciplinary research team to execute the clinical study according to the protocol. It is their prerogative to design the organization and select the personnel who will perform the functions. However, it is the sponsor's need for the Principal Investigator (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 ICH (International *Council for Harmonisation*) GCPs), 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 Health Risks (COFEPRIS) and in a platform of primary registries 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 FCI, the patient will receive a patient number with which all their information will be encrypted pseudonymity during collection and completely anonymized during analysis.

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

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

7.4 Outcome variables

7.4.1 Primary outcome variables

- OSDI score (TE: visits 1 and final).

7.4.2 Secondary outcome variables of efficacy

- Changes in the measurement of the TRLNI. (TE: visits 1 and final).
- Changes of corneal and conjunctival stains with lysmine green (ET: visits 1 and final).
- Corneal and conjunctival staining changes with fluorescein (ET: visits 1 and final).
- Conjunctival hyperemia changes (ET: visits 1 and final).

7.4.3 Safety variables

- Incidence of unexpected AEs related to the research product. (TE: Visits 1, Final and Safety Call)
- Changes in the MAVC (TE: visits 1 and final).
- Incidence of chemosis (ET: visits 1 and final).

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

Variable	Conceptual Definition	Operational Definition	Measurement Type	Normal value	Statistical test
OSDI **Score**	OSDI is a questionnaire designed to establish a severity and Classification of dry eye according to its symptoms.	The evaluator will apply the questionnaire to the subject and allow the subject to answer it calmly without any pressure and/or coercion. See annex 16.1 OSDI.	Continuous quantitative	Score: OSDI= (∑score)25 n answers answered	• ANOVA of repeated measures. • One-way ANOVA*
TRLNI	Measurement of tear rupture time using non- is based on the observation of the film's mirror reflection in a grating design or in the placid discs of the corneal topographers and their analysis using specialized software.	The TRLNI will be measured by means of the Oculus Keratograph 5M.	Discrete quantitative	>10s	• ANOVA of repeated measures. • One-way ANOVA*
Corneal and conjunctival staining with lysmine green	Detection of epithelial defects in the conjunctiva and cornea.	Direct observation with slit lamp, graduation on Oxford scale. See annex 16.2 Oxford scale.	Ordinal Qualitative	Degrees: The stain is presented in a series of panels (A-E). The points Stains range from 0-5 for each panel and from 0-15 for the exposed toral area of the conjunctiva and cornea.	• *X2* (Chi-square) of Pearson or Fisher's Exacta • McNemar Test*

Corneal and conjunctival staining with fluorescein	Detection of epithelial defects in the conjunctiva and cornea.	Direct observation with slit lamp, graduation on Oxford scale. See annex 16.2 Scale of Oxford.	Ordinal Qualitative	Degrees: The stain is presented in a series of panels (A-E). The points Stains range from 0-5 for each panel and from 0-15 for the exposed toral area of the conjunctiva and cornea.	• *X2* (Chi-square) of Pearson or Fisher's Exacta • McNemar Test*
Conjunctival Hyperemia Changes	It is defined as the simplest reaction of the conjunctiva to a stimulus, a red appearance is seen secondary to vasodilation of the conjunctival vessels of variable intensity.	Direct observation. Classification by Efron scale. See annex 16.3 Efron's scale.	Ordinal qualitative	Degrees: 0= Normal, 1= Very mild, 2= mild, 3= Moderate and 4= Severe	• *X2* (Chi-square) of Pearson or Fisher's Exacta • McNemar Test*
Incidence of chemosis	It is defined as conjunctival edema resulting from an inflammatory reaction. It is classified as present or absent.	The evaluator will use a narrow beam of light at 60° and measure whether the conjunctiva separates by ≥1/3 of the total palpebral aperture or whether it exceeds the gray line	Ordinal qualitative	Present/absent	• *One-way ANOVA* • *Pearson's X2 (Chi-square) or Fisher's Exact test*
Incidence of AEs	Any adverse medical event that occurs in a patient or clinical research subject who has been administered a pharmaceutical product and that does not	The AEs manifested during the conduct of the study will be collected using the FRCE.	Continuous quantitative Categorical qualitative	**Frequency** Subjects presenting EA/total number of subjects exposed. **Intensity** • Mild • Moderate • Severe **Causality**	*One-way ANOVA* *Pearson's X2 (Chi-square) or Fisher's Exact test*

				• Probable • Possible Unlikely	
Changes BCVA	Spatial VA is the ability to distinguish separate elements of an object and identify them. It is quantified as the minimum angle of separation (located at the nodal point of the eye) between two objects that allows them to be perceived as separate objects	Snellen chart	Discrete quantitative	**Fraction** **0.6 to 2.0**	*ANOVA for repeated measures.* *• One-way ANOVA**

7.4.4.1 Adverse events

An AE is any unfavorable medical appearance in a patient who is administered a PI, regardless of causal attribution.

Management of AEs will be performed as described in the Adverse Events section. See point 8 Evaluation and management of adverse events.

The PI will record in the corresponding section of the electronic case report form (FRCE) the AEs that the patients in the study may present, in addition to referring them in 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: the ophthalmological examination of the eyelids and adnexa; anterior and posterior segment that is performed in a routine ophthalmological examination, whose procedures are not specifically included in the study variables. Posterior pole evaluation can be with direct or indirect ophthalmoscopy, with or without pharmacological mydriasis, at the discretion of the PI. An assessment of the fundus will be carried out in search of abnormalities that alter the result of the study. IOP will be measured in this evaluation, with the PI choice instrument, it should be measured after the evaluation of stains. The result of the assessment will be recorded in the clinical file. In the electronic case report form (FRCE), only the findings that are considered so by the PI will be reported as AEs.

7.4.4.2 OSDI Score

OSDI is the most widely used symptomatic assessment tool in SOS clinical studies. It is referred to in the TFOS DEWS II as it is a diagnostic means already established among ophthalmologists.

It is a self-adpilating questionnaire of 12 questions, which is divided into three sections: frequency of symptoms, alteration of activities of daily living, worsening due to environmental factors in the last week. Each answer is assigned a numerical value which is then multiplied by 25 and then divided by the number of questions answered. The result can range from 0 to 100, classifying the severity of symptoms as specified below:

- < 12 = normal
- 13 – 22 = mild
- 23 – 32 = moderate
- ≥ 33 = severe

The OSDI score will be recorded in the FRCE.

Management as AE: A ≥ 40% increase in the baseline OSDI score value should be reported and managed as an AE due to lack of efficacy.

7.4.4.3 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. 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) equals 50 minutes. The Snellen fraction can also be expressed as a decimal (i.e., 20/20 = 1 and 20/40 = 0.5). [47]

VA will be evaluated at baseline, without refractive correction with the Snellen chart. It will be in a place with adequate lighting, natural or artificial, and at 3m from the patient to be evaluated. The visual acuity of each eye will be taken, starting with the right eye (OD) asking the patient to keep both eyes open and using an occlude to cover the left eye (OS); the patient 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 patient'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 FRCE, in addition to the FRCE it will be noted in decimal. CVAM cannot be inferior to VA.

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

7.4.4.4 TRLNI

For this study, it is required that the TRLNI be taken using the Oculus Keratograph 5M. This topographer has a high-resolution camera that allows the evaluation of the height of the tear meniscus, TRLNI and observation of meibomian glands.

The rings of Placido are reflected on the surface of the cornea, and when a distortion of the reflected sights occurs, it is recorded as a break in the tear film. The software then analyzes this information, and the results are expressed through a color-coded map. The red/orange segments correspond to a shorter rupture time, which will be evidenced in a map showing the total area of the cornea affected by tear film ruptures. The initial breakout time and average time will be displayed in the result presentation.

You will enable the "NIKBUT" button, then select infrared light. The patient will be asked to blink naturally, and when the device is aligned and the message "*Blink twice*" appears, it will prompt them to blink twice. After the second blink, the measurement will automatically start. The patient should keep their eyes open without blinking, as the measurement is automatically interrupted if the patient blinks, moves a lot, or the tear film breaks significantly.

As previously mentioned, a TRLNI of less than 10 sec. Viewpoint

The value of the first break-up time will be recorded in the FRCE under TRLNI. This study may be carried out within a window of 0 to 2 days between the screening visit and the baseline visit, prior to the latter; as well as ± 2 days with respect to visits 1 and final. It must be carried out at least 2 hours after having applied any stain or drug allowed by this protocol for ophthalmic check-ups.

7.4.4.5 Corneal and conjunctival fluorescein staining

A drop of topical anesthetic will be instilled in the bottom of the conjunctival sac, then a second drop will be applied to the tip of the fluorescein strip, allowing it to sit on the strip for 5 seconds to elute the dye, shaking off the excess at the end. A small contact of the strip is made with the conjunctiva at the fundus of the temporal sac, while the patient looks upwards, without damaging the conjunctiva. It will be graded according to the Oxford scale. [48]

The PI will record in the file and the FRCE the grade granted 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.

7.4.4.6 Conjunctival staining with lysmine green

After the revision with fluorescein, a drop of saline solution will be applied to the tip of the green lysam 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. [48]

The PI will record in the file and the FRCE the grade granted 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.

7.4.4.7 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. It will be graduated using the Efron scale. [49] See annex 16.3 Efron's scale.

Management as AS: conjunctival hyperemias classified as grade 3 or higher will be considered as AD.

7.4.4.8 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. [50] Management as an EA: its presence will be considered as an EA.

7.5 Study visit and activities program

7.5.1 Description of activities per visit

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

7.5.1.1 Counting 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 patient's health conditions, medical acts and other procedures performed on the patient 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 patient is taken from the established population base of the study center, the existing medical history may be used, and only one update must be made.
- Evaluation of concomitant medications refers to the questioning by the PI of the patient, 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 IP 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.
- OSDI: see section 7.4.4.2 OSDI score.
- CVAM: see section 7.4.4.3 Better corrected visual acuity.
- Ocular surface stains: see points 7.4.4.5 Corneal and conjunctival staining with fluorescein and 7.4.4.6 Conjunctival staining with lysmine green.
- Comprehensive ophthalmological evaluation: see point 7.4.4.1 Adverse events.
- Eligibility criteria: refers to the review by the PI, where it is found that the patient can be included in the study by meeting the inclusion criteria and not meeting the exclusion criteria.
- Evaluation of AEs: see section 7.4.4.1 Adverse events.

It will be at the discretion of the principal investigator whether the clinical evaluations that will be considered the baseline values of each variable will be those evaluated during the screening visit, or if he or she considers it necessary to repeat any test during the baseline visit. In the latter case, these results should be considered as baseline values.

7.5.1.2 Basal Visit

- Eligibility criteria: refers to the review by the PI, where it is found that the patient can be included in the study by meeting the inclusion criteria and not meeting the exclusion criteria.
- Evaluation of AEs: see section 7.4.4.1 Adverse events.
- TRLNI: see point 7.4.4.4 TRLNI.

- IP randomization: refers to determining the intervention that the patient will follow during the study. This assignment will be made at baseline visit (day 0). See point 7.3 Randomization
- Delivery of the PI and initiation of intervention refers to the delivery of the PI to the study patient, by the research center.
- Delivery of patient material and filling instructions refers to the delivery by the PI to the patient of the patient's diary, ID card and calendar improving adherence. The staff assigned by the research center will carry out prior training to the patient, on the completion of the diary.

7.5.1.3 Visit 1

- Evaluation of concomitant medications: see point 7.5.1.1 Screening Visit.
- 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.
- OSDI: see section 7.4.4.2 OSDI score.
- CVAM: see section 7.4.4.3 Better corrected visual acuity.
- TRLNI: see point 7.4.4.4 TRLNI.
- Ocular surface stains: see points 7.4.4.5 Corneal and conjunctival staining with fluorescein and 7.4.4.6 Conjunctival staining with lysmine green.
- Comprehensive ophthalmological evaluation: see point 7.4.4.1 Adverse events.
- Evaluation of AEs: see section 7.4.4.1 Adverse events.
- Evaluation of adherence: see point 7.5.1.1 Scrutiny visit.

7.5.1.4 Final Visit

- Evaluation of concomitant medications: see point 7.5.1.1 Screening Visit.
- Urine pregnancy test: see point 7.5.1.1 Screening Visit.
- Vital signs: see point 7.5.1.1 Scrutiny visit.
- Evaluation of AEs: see section 7.4.4.1 Adverse events.
- OSDI: see section 7.4.4.2 OSDI score.
- CVAM: see section 7.4.4.3 Better corrected visual acuity.

- TRLNI: see point 7.4.4.4 TRLNI.
- Ocular surface stains: see points 7.4.4.5 Corneal and conjunctival staining with fluorescein and 7.4.4.6 Conjunctival staining with lysmine green.
- Comprehensive ophthalmological evaluation: see point 7.4.4.1 Adverse events.
- Evaluation of the Patient's Diary: see point 7.5.1.1 Screening Visit.
- Evaluation of adherence: see point 7.5.1.1 Scrutiny visit.
- Return of PI and patient diary: refers to the return by the patient, the PI and the patient's diary to the research site.

7.5.2 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 AE reporting. During these visits, all pertinent data on the AEs reported should be collected and, where appropriate, an appropriate management plan should be established.

7.6 Data collection

7.6.1 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.

7.6.2 Electronic forms of data collection

All data related to the protocol will be captured through an eCRF by the research team staff. The data related to the protocol should NOT be captured directly in the eCRF but should be transcribed from the corresponding source document. This procedure allows monitoring to verify the information captured in the eCRF. It is the responsibility of the researcher to ensure that the information is transcribed to the eCRF in a correct, complete, and timely manner. It is understood that all FRCEs captured and submitted for data analysis are approved by the Investigator.

7.6.3 File

The data collected in this database is anonymous (it only stores the patient number together with other information of interest). The program used for data capture and storage covers the traceability requirements necessary for the execution of clinical studies. The data collected will be stored by the sponsor or the clinical research organization designated for this purpose and its storage will have a duration of 10 years. The 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.

7.6.4 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 AE reporting. During these visits, all pertinent data on the AEs reported should be collected and, where appropriate, an appropriate management plan should be established.

8. Evaluation and management of adverse events

8.1 Regulation and regulations on adverse events

The registration and reporting of AEs will be carried out in accordance with the guidelines established in NOM220-SSA1-2016, which is in accordance with the international guidelines ICH E6.

8.2 Definition 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 EA can be any of the following: any undesirable medical event 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.

8.3 Definitions relevant to the classification of adverse events

Severity (serious/non-serious), also called seriousness (serious/not serious). Serious or serious is defined as any event that: results in death, threatens life, requires hospitalization or prolongs hospitalization, is a cause of permanent or significant disability or disability, is the cause of alterations or malformations in the newborn, other medically important conditions.

Severity (mild, moderate, or severe). Mild is those that present with minimal symptoms, do not require treatment or suspension of the medication; moderate, when they interfere with usual activities, without threatening the 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; unclassifiable, those for which, once all possible information about the EA has been obtained, it remains unclassifiable.

8.4 Responsibilities of the researcher

It is the responsibility of the Investigator to verify AEs through questioning, review of the information recorded in the patient'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. [51] [52] [53]

In the event of AEs or any event that puts the health and well-being of patients at risk, relevant medical care will be provided, either at the research center or referred to the Hospital Center with the greatest 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, it will notify the sponsor and register the corresponding information in the FRCE and in turn, inform the IEC and IC.

The attention of the AEs will be carried out according to the event attention diagram. See Figure 6 Adverse Event Care.

The final report to be written by the Clinical Team of the Medical Management Department of Laboratorios Sophia, S.A. de C.V., will include the report of the AEs in compliance with current national and international regulations. [52] [51]

If the research patient debuts during their participation in the study with a 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.

8.4.1 Record of adverse events in the electronic case report form

EA registration considers:

- The patient's identifying information such as: code, age, gender, left eye, right eye.
- Information about the type of AE to the PI or the drug under study, as appropriate.
- Important date information:
    - Date the EA 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 the PI and/or investigational drug 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 AEs.

Record the outcome or resolution of the event:

- Recovered patient without sequelae
- Recovered patient with sequelae
- Patient not recovered
- Patient who died due to AE
- Patient who died and it is judged that the drug may have contributed
- Patient who had death and it is not related to the product or drug under investigation,
- Or indicate that you do not know what the consequence of the event is.

– Information about the investigational product or drug or the drug associated with the EA, 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 blinded 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 investigational patient 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 investigational drug, PI, or drug, as appropriate. Indicate whether withdrawing the PI or investigational drug or suspected drug (of causing the event) makes the EA disappear. 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 patients who are exposed again to the PI, investigational drug or drug, which had previously been discontinued, the AD reappears.

– Information regarding concomitant pharmacotherapy. Indicate the generic name, dose, route of administration, start and end dates of use, as well as the reason for the prescription, regardless of whether it is in accordance with the prescribing information or technical data sheet or is used outside the regulations or what has been authorized by the local, national or international regulatory entity.

- Relevant medical history information. The analysis of the EA 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.

8.4.2 Adverse Event Tracking

The PI will provide the attention and management of the EA presented by the participant until the outcome of the same, according to what is referred to in the following section.

8.4.3 Procedures for a serious adverse event

The AE care process considers the following stages:

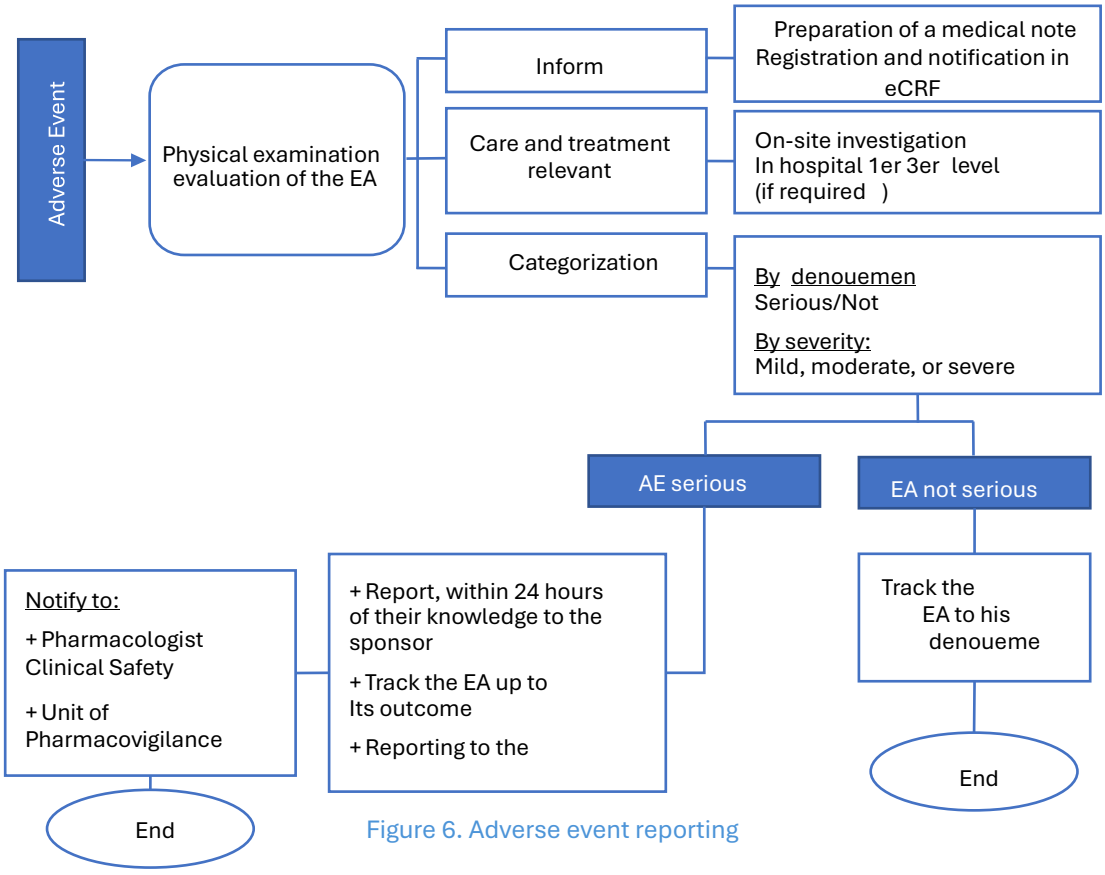


Figure 6. Adverse event reporting

During the development and conduct of this study, undesirable harmful events or adverse reactions of medical implication may occur in the investigational patient, which do not necessarily have a causal relationship with the PI or investigational drug. 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 PI or the investigational drug causes some unwanted clinical manifestation. AEs, ADRs or SRAMs 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 referral can be with a card that identifies the patient 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 EA is associated with or is in relation 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 FRCE and its respective EA section. By, in cases of severe AEs, which must be notified in less than 24 hours after the PI becomes aware of it, the clinical monitor of the study is informed, so that in turn it informs the Clinical Team and the Pharmacovigilance and Technovigilance Unit of Laboratorios Sophia (UTFLS), so that it can later inform the CEI. With respect to non-serious AEs, they will be registered and treated appropriately, and the corresponding regulatory entity will be informed about the safety profile of the PI or investigational drug 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 patient, since it is expected that most of the harmful phenomena (see section of the safety profile in 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.

8.4.4 Causation assessment

Causality assessment is the methodology used to estimate the probability of attributing the observed AE to a drug, investigational drug or IP. It considers probabilistic categories according to the available evidence and the quality of the information, based on national pharmacovigilance regulations. [51]

The UFTLS can use the algorithm of Karch and Lasagna modified by Naranjo referred to by Aramendi I, as a tool to facilitate the probabilistic categorization of causality. In this algorithm, different items are scored, which allow assigning a value to the cause-effect relationship between the administration of the drug and the adverse reaction. [54] See Appendix 16.4 Karch and Lasagna algorithm modified by Naranjo.

In such a way, the degree of certainty to establish the PI or investigational drug (as appropriate) as the causal agent of the harmful phenomenon that occurs to the patient 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 adverse event.
e. Experimental findings, e.g., the appearance of abnormal metabolites or high levels of the drug or its biotransformation product.
f. Analogy, which refers to the experience gained with other related drugs, adverse reactions frequently produced by the same family of pharmacological agents.
g. Nature and characteristics of the data, i.e. objectivity, accuracy and validity of the relevant documentation. [55]

8.5 Unanticipated Issues

Unanticipated problems (APNs) consider those situations that pose risks to participating patients, in general, any incident, experience or outcome 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.

8.5.1 PNA Report

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

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

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

Any other NAPs will be reported to the CEI and the sponsor within the first 5 business days after the PI became 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 visits and one closing visit must be carried out, which does not exclude the carrying out of one or more follow-up visits between these two mandatory visits.

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

At the follow-up visit(s), the monitor will conduct a review of the study documents to confirm that: the research protocol has been followed, the applicable standard operating procedures have been followed, the data has been completed and timely, and that the AE reports have been carried out appropriately. During this visit, the monitor will discuss the findings with the researcher and define the actions that should be taken.

The closure visit will take place at the end of the study, once the last site participant has been discharged from follow-up. On this visit the monitor will verify that the site has all the necessary documents for archiving, that all biological samples have been sent for analysis, that all the drug under study (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 relevant 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.

9.2.1 Pre-study audit

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

9.2.2 Auditing during the conduct of the study

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

10. 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 SPSS statistical package version 19.0 for Windows (IBM Corporation, Armonk, NY, USA) will be used. The coding will be done using consecutive numbers. The data will be collected and sorted in an Excel spreadsheet (Microsoft® Office). The data will then be exported to the SPSS package platform. The variables will be categorized according to their nature. See point 7.4.4 Definition of variables, methods and scales to be used for their measurement.

10.1.2 Data Interpretation

The Kolmogorov-Smirnov and Shapiro Wilk tests will be performed, as applicable, to determine if the distribution of the data is normal in the results obtained in each study group [56].

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

- Intra-group analysis: For equal variances, Tukey's POST-HOC test will be used, otherwise the Dunnett statistic will be used. One-way analysis of variance (one-way ANOVA) will be used when applicable.
- Intergroup analysis: Differences between groups will be analyzed using the Repeated Measures Analysis of Variance (ANOVA) test or the Student's t-statistic when applicable.

A non-inferiority margin (δ) of 10% will be used for the primary efficacy endpoint, based on the primary endpoint of the study. The level of difference to consider significance will be an alpha (α) of 0.05 or less.

The result of the nominal and ordinal qualitative variables will be presented in frequencies, proportions and/or 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) [7].

- Difference between groups: Pearson's X2 (Chi-square) test 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 adverse events, all subjects who were randomly assigned to an intervention group after baseline (intention-to-treat population, ITT) will be considered.

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

Variable Type	Variable	A1	B1	B2	C1	D1	D2	D3	D4	E1	E2
Background											
A1	Demographic	DT									
Basal											
B1	Medical History/Selection Criteria	DT									
B2	Comprehensive ophthalmological evaluation	DT				TB	T	T	T	TB	TB
Efficiency											
C1	OSDI	DB			B					TB	
Secondary efficacy outcome											
D1	TRLNI				DB	B				TB	
D2	Corneal and conjunctival stains (TVL and TF)		DT	DT			TB			TB	
D3	Conjunctival Hyperemia		DT	DT			TB			TB	
D4	Chemosis		DT	DT			TB			TB	
Safety											
E1	Incidence of AD				TB	TB	TB	TB	TB	TM	
E2	MAVC		DB							TM	B

D, Descriptive Statistics; T, 2x2 contingency table; B, Bivariate Analysis; M, Multivariate Analysis.

Table 3. Triangulation of concepts

10.1.3 Procedure for handling missing data

The safety assessment will include in the analysis all those patients (both eyes) who have been exposed at least once to the intervention, regardless of the visit at which they were eliminated from the study (ITT).

10.1.4 Deviations from the statistical analysis plan

According to the calculation of the sample size to meet the objective of the study, 50 evaluable patients are required per arm. If this number is not met due to a loss of patients greater than 20% contemplated in this protocol (loss of follow-up or withdrawal of ICF), the sponsor may replace these patients.

The results obtained from the replaced patients will continue to be used for safety analysis and will be part of the ITT.

10.1.5 Patients included in the analysis

Those patients who meet an adherence ≥65% of the expected weight of the PI and ≥80% of expected applications in the patient's diary, will be included in the statistical analysis to meet the objective of the study. If it is not possible to assess the weight of the IP, only the patient's diary will be considered.

10.2 Sample Size Calculation

10.2.1 N Calculated

Number of planned patients: 60 evaluable patients (both eyes) per arm

Total N = 180 patients

10.2.2 Justification for sample calculation

Sample size calculation was performed based on the results of the phase IV, multicenter, prospective, double-blind, randomized efficacy and safety clinical study of Humylub Ofteno® PF ophthalmic solution, versus Systane® Ultra and Systane® Ultra preservative free performed in subjects diagnosed with mild to moderate dry eye. The reduction in the mean score of the OSDI test was considered as the main efficacy variable, with a dosage of 1 drop QID. In this study, after one month of treatment, subjects assigned to Humylub Ofteno® PF had a decrease of-49.3% vs-41.3% for Systane Ultra and-48.3% for Systane Ultra PF, with respect to their initial mean values with no statistical differences between treatments (p>0.05) [57].

For the present protocol, it is expected that, after 3 days of treatment, 30% of subjects treated with Humylub Ofteno® PF will have a 35% decrease in OSDI score compared to their initial mean value.

The sample size was calculated using the equation for two proportions [58], considering a power of 80% (β), a significance level of 0.05 (α) and a margin of non-inferiority (δ) of 10% and 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:

$$k = \frac{\eta A}{\eta B}$$

Equations

The calculation to estimate sample size and power was performed using an online tool and following the equations: [59]

$$n_A = kn_B \; y \; n_{B=}\left(\frac{P_A(1-P_A)}{k} + P_B(1-P_B)\right)\left(\frac{z_{1-\alpha} + z_{1-\beta}}{p_A - p_B - \delta}\right)^2$$

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

k=nA/nB

Φ is the standard normal distribution function

Φ^{-1} is the standard normal quantile function

α is a Type I error,

β is the Type II error, which means that 1-β is the power, and

δ is the test margin.

According to the previous calculation, the result was 50 subjects, this calculation was increased by 20% considering possible losses (10 subjects). The total suggested sample size is 180 subjects (60 cases per arm), which will provide both eyes for the study.

11. Ethical considerations

11.1 Approval of the committees

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 ICH, GCP Guide. Council for International Organizations of Medical Sciences (CIOMS, 2016) International Ethical Guidelines for Biomedical Research in Human Subjects. The Research Ethics Committee and the Research Committee will evaluate the protocol before conducting the study and will issue their approval or possible modifications for its performance, these Committees must be notified of any significant changes to the protocol. In addition to the above, the current regulations of the regulatory authority will also be complied with.

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

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

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

The study is a study with greater than the minimum risk, according to

Regulations of the General Health Law on Health Research, Title Two, Chapter I, Article 17, Section III, published in the Official Gazette of January 6, 1987.

11.2 Amendments to the protocol

The amendment procedure will be relevant when there is a need to make any change to a document that is part of the research project or protocol, derived from variations in the methodological structure, substitution of the principal investigator or in the face of the identification of risks in the patients who will participate in the research. 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 Research Ethics Committee and the Research Committee or, where applicable, by the Biosafety Committee, (entities that issued the initial favorable opinion for the conduct of the research) will be sent for authorization by the relevant regulatory entity.

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

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

11.3 Early Study Termination

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

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

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

   economic support, manufacturing errors, etc.
4. Lower recruitment than stipulated.
5. Identification of unexpected risks to participants, which are significant or unacceptable.
6. Obtaining new relevant safety information.
7. Insufficient adherence to the requirements of the protocol.
8. The data obtained are not assessable or are not sufficiently complete.
9. Determination that the primary objective has been achieved.

10. Determination of futility.

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

11.4 Informed Consent

The FCI contains complete and understandable information about the study and the product under investigation, in accordance with the applicable regulations in force and the GCP.

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

11.4.1 Obtaining

Informed consent must be obtained before the patient 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 GCPs and will be following 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 patient, it will be explained to the patient that he or she has the right to interrupt his or her participation in the study at any stage, without affecting the relationship with the investigator and/or his or her future care. Informed consent will be put to the consideration of the potential participant; He must have enough time to analyze 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 may or may not be related to the study patient, who will participate during the informed consent process and 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.

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

The FCI 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.

11.4.2 Special considerations

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

11.4.3 Modifications to informed consent

Any changes to the FCI constitute an amendment to this document and must be submitted for approval to the Research Ethics Committees, and if applicable to the Competent Authorities.

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

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

11.5 Confidentiality

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

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 if 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 team to obtain informed consent from patients 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 patient selection record, as well as the identification and enrollment list of each of the patients 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 FRCE and communications related to study patients will identify them only by the study patient 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 CEI, the IC, the sponsor, the monitors/auditors and third-party auditors will be the only bodies authorized to review the study documentation. If publications arise from this research project, in no case will they contain information on the identification of the patients in the study. If the results of the study are published, no personal information of patients will be revealed.

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

11.6 Conflict of interest

The independence of the conduct of the study and its results from any current or perceived external influences is critical. For this reason, any current conflict of interest of any person who has a role in 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.

11.6.1 Declaration of Interests

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

11.7 Access to Information

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

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

11.8 Ancillary and post-study care

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

12. Biosecurity aspects

NO BIOSECURITY IMPLICATIONS

The present protocol, entitled: "Phase IV non-inferiority clinical study, to compare the efficacy of the ophthalmic solution Humylub Ofteno® PF against Hyabak® and Lagricel Ofteno® PF, on the ocular surface of patients with mild to moderate dry eye syndrome.", and number: SOPH087-0120/IV HAS NO BIOSAFETY IMPLICATIONS, since infectious and contagious biological material will NOT be used; pathogenic strains of bacteria or parasites; viruses of any kind; radioactive material of any kind; genetically modified animals and/or cells and/or plants; toxic, dangerous or explosive substances; any other material that puts the health or physical integrity of the research centre staff or patients at risk 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. Posting 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 Department of Laboratorios Sophia, S.A. de C.V. This report will be prepared following the recommendations of the E3 Step 4 Guide of the ICH.

13.2 Communication of results

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

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

13.3 Publication of results

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

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

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

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

14.Financing and Insurance

14.1 Compensation to Study Participants

Patients who participate in the study will not receive financial compensation for their participation in the study. However, randomized patients will receive financial support for travel expenses at each scheduled visit 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

Patients 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

OSDI (Ocular Surface Disease Index)

Identification card

No. of Study: SOPH087-0120/IV **Date:** __/____/____

Subject's initials: _____ **Subject No.:** 087-_____-____

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 all moment	**Almost everywhere moment**	**50% of the time**	**Almost nowhere 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

Over the past week, Have you felt discomfort in your eyes with any of the following situations?	Frequency					
	In all moment	**Almost everywhere moment**	**50% of the Time Moment**	**Almost in no**	**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

(B)

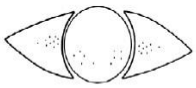
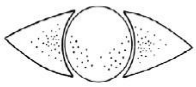
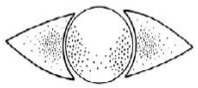
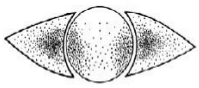
Over the past week, Have you had eye problems that have limited you from performing these actions?	Frequency					
	In all moment	**Almost everywhere moment**	**50% of the time**	**Almost nowhere moment**	**In no moment**	**NA**
6. Read	4	3	2	1	0	NA
7. Driving at night	4	3	2	1	0	NA
8. Working with a computer or an ATM	4	3	2	1	0	NA
9. Watch TV	4	3	2	1	0	NA

Sum of A+B+C = (D)

Subtotal of the answered cells (C)

Number of items answered, do not include responses as NA (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 the A
C 	II	Equal to or less than panel C, greater than B
D 	III	Equal to or less than panel D, greater than C
E 	IV	Equal to or less than panel E, greater than D
>E	V	Larger than panel E

16.3 Efron's Scale

16.4 Karch and Lasagna's algorithm modified by Naranjo

No.	Question	Score	
		Yes	No

1	There are conclusive previous reports of adverse drug reactions, adverse events, or suspected adverse drug reactions.	+1	0
2	The adverse event occurred when the suspected drug was administered.	+2	-1
3	The adverse drug reaction, adverse event, or suspected adverse drug reaction improved upon discontinuation or administration of a specific antagonist.	+1	0
4	The adverse drug reaction/adverse event/suspected adverse drug reaction reappeared when the drug/investigational product/investigational drug was administered	+2	-1
5	There are alternative causes that may trigger this reaction.	-1	+2
6	The adverse reaction/adverse event/suspected adverse drug reaction occurred after administration of placebo.	-1	+1
7	The drug was detected in the blood or other fluids in toxic concentrations	+1	0
8	The intensity of the adverse reaction/adverse event/suspected adverse drug reaction was greater with higher doses or lower with 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	The adverse reaction/adverse event/suspected adverse drug reaction was confirmed by some objective evidence	+1	0
	Total score		
Probabilistic category based on the score obtained			
I	The causal relationship is verified.	≥ 9	
II	The RAM is likely due to the investigational drug or product.	5 to 8	
III	It is possible that the RAM is due to the investigational drug or product.	1 to 4	
IV	The causal relationship is questionable.	0	
Each reagent receives a defined score, and the final sum allows for an estimate of the probabilistic category of the cause-effect relationship between the administration of the investigational product and the adverse reaction, adverse event, or suspected adverse reaction			