Excelencia en oftálmicos

Study protocol:

SOPH037-0120/IV

Title: Phase IV clinical study to evaluate the efficacy of Lagricel® Ofteno PF compared to Thealoz® Duo in corneal reepithelialization following PRK (Photorefractive Keratectomy)

Information about the study molecule

Generic name: Sodium hyaluronate 0.4%

Distinctive name: Lagricel® Ofteno PF

Indication: Eye lubricant

Protocol information

Phase of the study: IV

Clinical Trial Code: NCT04704518

Version date: January 26th 2022

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

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

Content

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Study leaders

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

Table 1. Study leaders

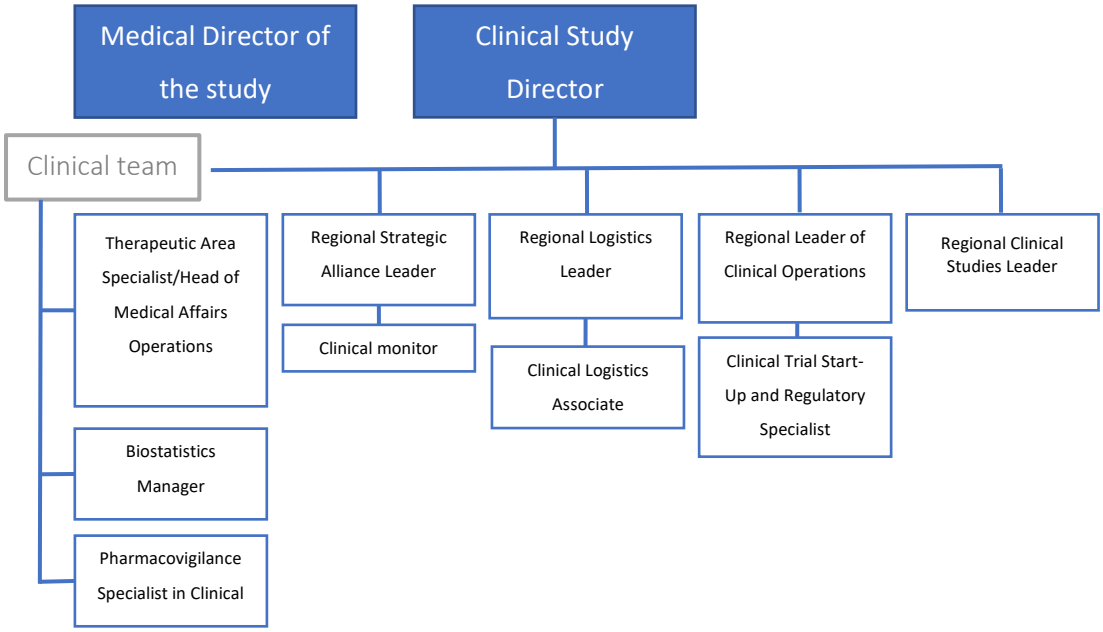
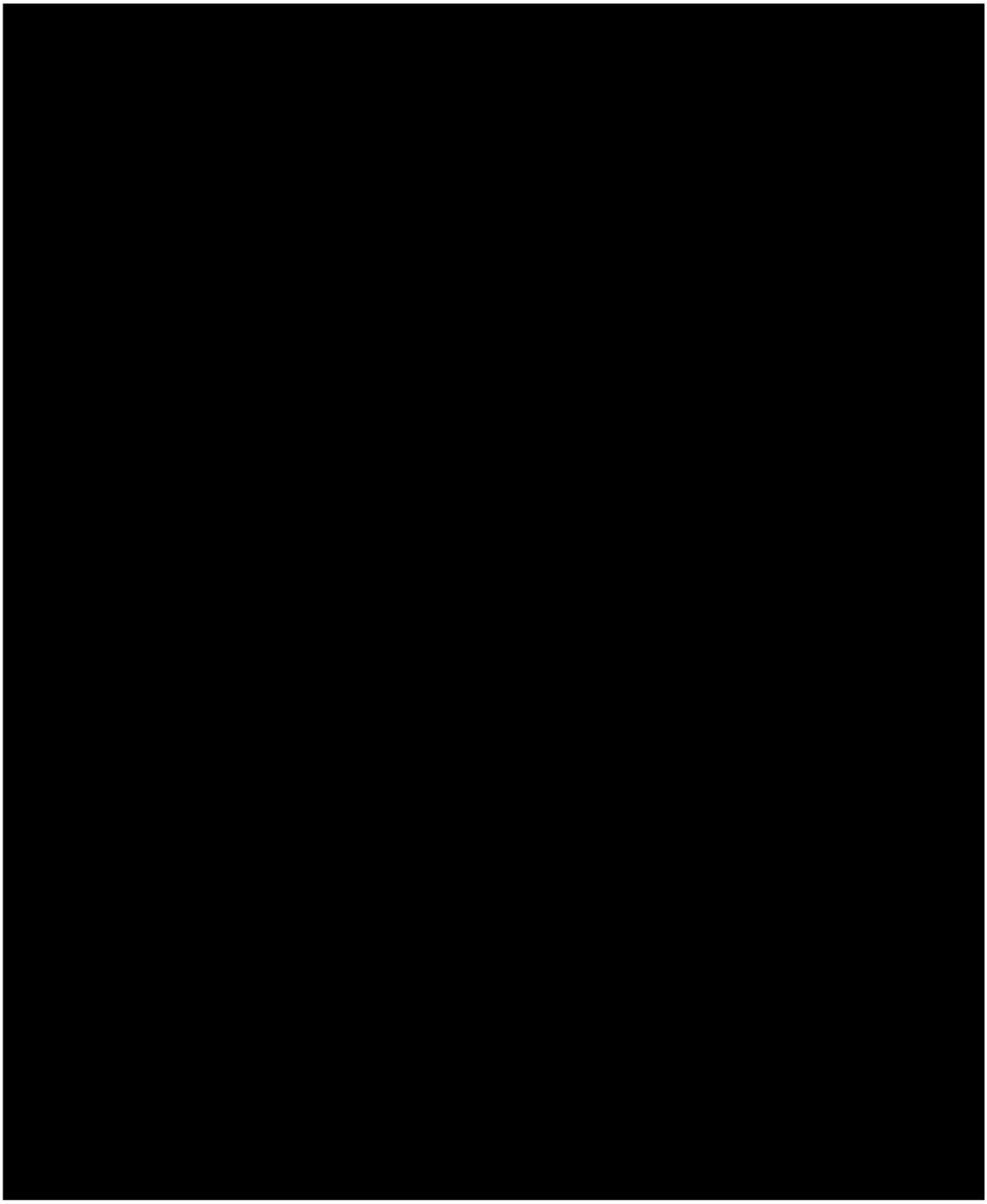


Figure 1. Administrative structure

Signature pages



Researcher Agreement

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

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

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

List of abbreviations

AE	Adverse event
CI	Confidence interval
COFEPRIS	Federal Commission for the Protection against Sanitary Risks
CONSORT	Consolidated Standards of Reporting Trials
D	Day
eCRF	Electronic Case Report Form
FDA	United States Food and Drug Administration Administration
IC	Investigation Committee
ICF	Informed consent form
ICH	International Council for Harmonization
ICMJE	International Committee of Medical Journal Editors
ICO	Eye comfort index
IEC	Independent Ethics Committee
IP	Investigational Product
ITT	Intention-to-treat population
IUD	Intrauterine device
n	Number
NIBUT	Non-invasive tear film break-up time
NRCT	National Registry of Clinical Trials
OD	Right eye
OSD	Ocular surface disease
OU	Both eyes
PEE	Punctate epithelial erosion
PI	Principal Investigator
PP	Per-protocol population
PRK	Photorefractive keratectomy
PRN	*Pro re nata*
QID	Four times a day (*quater in die*)
RA	Rheumatoid arthritis
SC	Safety call

SH	Sodium hyaluronate
SICCA	Sjögren's International Collaborative Clinical Alliance
SLE	Systemic lupus erythematosus
TBUT	Tear film break-up time
TEAE	Evaluation time
TID	Three times a day (*ter in die*)
UP	Unanticipated problems
VA	Visual acuity
BV	Baseline visit
V1, V2, V3	Follow-up visits
FV	Final visit
WHO	World Health Organization

1. Summary

1.1 Synopsis

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

Phase IV clinical study to evaluate the efficacy of Lagricel® Ofteno PF compared to Thealoz® Duo in corneal reepithelialization following PRK (Photorefractive Keratectomy)</td></tr>
<tr><td>Study number:
SOPH037-0120/IV</td><td>Creation date:
02-Jan-20</td></tr>
<tr><td>Protocol version:
Clinical Trial Code: NCT04704518</td><td>Version date:
January 26, 2022</td></tr>
<tr><td>Therapeutic indication:
Eye lubricant</td><td>Use:
Corneal reepithelialization</td></tr>
<tr><td>Estimated duration of the study (from the first visit of the first patient to the preparation of the final report) :
11 months</td><td>Clinical development phase: IV</td></tr>
<tr><td colspan="2">Goals:

Main objective:

- To evaluate the efficacy of Lagricel® Ofteno PF compared to Thealoz ® Duo in corneal reepithelialization of post-PRK surgery patients.

Specific objectives

- To evaluate the safety of Lagricel® Ofteno PF compared to Thealoz® Duo by the incidence of unexpected adverse events (AEs) related to the investigational product in post-PRK surgery patients .
- To determine the efficacy of the Lagricel® Ofteno PF formulation compared to Thealoz® Duo in corneal reepithelialization of post-PRK surgery patients by means of corneal fluorescein staining by direct observation and photographic record .
- To determine the efficacy of the Lagricel® Ofteno PF formulation compared to Thealoz® Duo in corneal reepithelialization of post-PRK surgery patients using the ICO test .
- To determine the efficacy of the Lagricel® Ofteno PF formulation compared to</td></tr>
</table>

Thealoz® Duo in corneal reepithelialization of post-PRK surgery patients by assessing pain perception.

- To determine the safety of Lagricel® Ofteno PF formulation compared to Thealoz® Duo in corneal reepithelialization of post-PRK surgery patients through changes in visual acuity .

Hypothesis :

H_0: Lagricel® Ofteno PF does not contribute to complete corneal reepithelialization at the 3rd day of post-PRK treatment and is not equivalent to Thealoz® Duo .

$$H_0: |\mu_A - \mu_B| \geq \delta$$

H_1: Lagricel® Ofteno PF contributes to a reepithelialization corneal complete to the 3rd day ofpost-surgical treatment of PRK being equivalent a Thealoz® Duo .

$$H_1: |\mu_A - \mu_B| < \delta$$

Study design:

A Phase IV, multicenter, comparative, controlled, randomized, open-label, parallel-group study.

Number of subjects (planned and analyzed):

Number of planned subjects: 96 evaluable patients

- Both eyes (OU): safety assessment
- Right eye (RO): efficacy assessment
  48 evaluable patients per group (2 Groups)

Diagnosis and main inclusion criteria:

- Patients in the 1-day post-PRK surgery period.

Selection criteria:

Inclusion criteria:

- Be between 18 and 45 years old.
- Period of 1st day post-surgical of PRK (HE will evaluate for effectiveness eye right).
- Having performed the epithelium removal technique with 20% alcohol (ethanol) for a time within the range of 30-40 seconds.
- Having used a de-epithelialization area (transition area) in the range of 8-9 mm.
- Surface of ablation corneal surgical of 5.5 to 6.0 mm.
- Corneal expenditure less than or equal to 60 microns.
- Refraction pre-surgical of -1.0 to -4.5 D of myopia either astigmatism myopic (the

addition of both values in this case, equivalent spherical no elderly to -4.5).
- Have the ability of grant of shape voluntary his consent informed signed.
- Be able and willing to comply with scheduled visits, treatment plan, and other study procedures.
- Be willing to modify the activities of your style of life. See 5.3 Lifestyle considerations
- Women of childbearing age must ensure continuation (started ≥ 30 days prior to signing) of the Shape of Consent Informed [ICF]) of use of a method contraceptive hormonal either device intrauterine (IUD) during he period of the study.

Exclusion criteria:

- Transurgical complications and during the post-surgical period prior to inclusion in the study.
- Use of mitomycin during PRK.
- PRK retreatments or history of other types of refractive surgery.
- For women: be pregnant, breastfeeding, or planning to become pregnant during the study period.
- Having participated in another clinical research study ≤ 30 days prior to the screening visit.
- Having previously participated in this study.
- Present an additional diagnosis of:
    - Allergic, viral or bacterial conjunctivitis.
    - Dry eye.
    - Anterior blepharitis.
    - Parasitic eye infections (e.g., *Demodex*).
    - History of ocular herpes.
    - History of ocular inflammatory processes (such as uveitis).
    - History of corneal or conjunctival ulcers.
    - Glaucoma.
- Have a history of drug addiction or dependence, currently or within the last two years prior to signing the ICF.
- Have a history of ocular surgical procedures within the last 3 months prior to signing the ICF.
- Having another medical condition, acute or chronic (such as Diabetes Mellitus type I / II, autoimmune diseases or HIV) that, in the opinion of the investigator, could increase the risk associated with participation in the study or the administration of the investigational product, or that could interfere with the interpretation of the study results.
- Use medications (such as retinoic acid) that, in the opinion of the investigator, 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 study results.
- Have a known hypersensitivity to the components of the investigational products.
- Be or have an immediate family member (e.g., spouse, parent/legal guardian, sibling, or child) who is an employee of the research site or the sponsor and who is directly involved in this study.

Research Product:

Lagricel® Ofteno, multidose presentation. Sodium hyaluronate 0.4% ophthalmic solution.

- Manufacturer: Laboratorios Sophia, S.A. de C.V.
- Posology: Group 1, 1 drop QID, OU.
- Route of administration: Ophthalmic topical.

Thealoz® Duo, Trehalose 3% / Sodium hyaluronate 0.15% ophthalmic solution.

- Manufacturer: Laboratorios THEA.
- Posology: Group 2, 1 drop QID, OU.
- Route of administration: Ophthalmic topical.

Duration of treatment:	**Subject's duration in the study:**
14 days	Up to 18 days

Evaluation criteria:

Primary efficacy outcome variable:
- Corneal re-epithelialization time (TEAE: Day 1, 2, 3, and 7).

Secondary efficacy outcome variables:
- Percentage of postoperative corneal re-epithelialization (TEAE: Day 1, 2, 3, and 7).
- Test score (TEAE: Day 1, 7, and 15).
- Pain perception assessment (TEAE: Day 1, 2, 3, 7, and 15).

Primary safety outcome variable:
- Incidence of AE (TEAE: Day 2, 3, 7, and 15).
- Changes in VA (TEAE: Day 2, 3, 7, and 15).

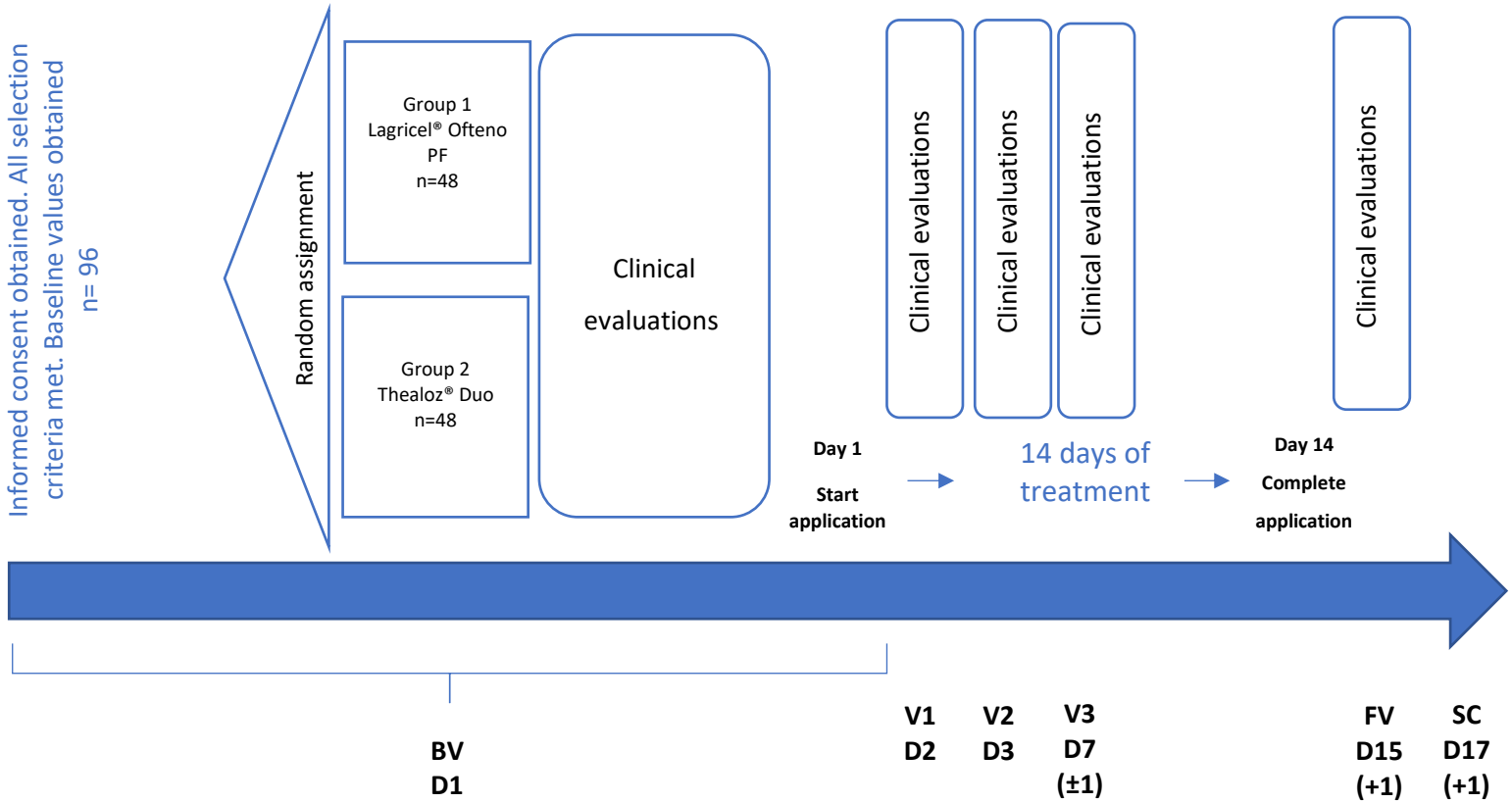
Statistical methodology

The Shapiro–Wilk test will be performed to assess whether the distribution of the results in each study group follows a normal distribution. Quantitative variables will be summarized using measures of central tendency, including mean and standard deviation. Qualitative variables will be presented as frequencies and percentages.

For comparisons between groups, quantitative variables will be analyzed using the Student's t-test. If the assumption of normality is not met, the Mann–Whitney U test will be used. Differences between qualitative variables will be analyzed using the Chi-square (χ^2) test or Fisher's exact test, as appropriate.

A two-sided significance level (α) of ≤ 0.05 will be considered statistically significant.

1.2 Study diagram



VB = Baseline visit, V1 = Follow-up visit 1, V2 = Follow-up visit 2, V3 = Follow-up visit 3, VF = Final visit, SC = Safety call, D = Day.

(Allowed window period)

Figure 2. Diagram of the study.

1.3 Study schedule

PROCEDURES	BV D1	V1 D2	V2 D3	V3 D7 ±1	FV D15 ±1	SC D17 ±1
ICF SIGNATURE	X					
MEDICAL RECORD	X					
CONCOMITANT DRUG EVALUATION	X	X	X	X	X	
URINE PREGNANCY TEST	X				X	
VITAL SIGNS	X	X	X	X	X	
VA	X	X	X	X	X	
EVALUATION OF CORNEAL REEPITHELIALIZATION (BY PHOTOGRAPHIC RECORD, USING FLUORESCEIN)	X	X	X	X		
COMPREHENSIVE OPHTHALMOLOGICAL EVALUATION	X	X	X	X	X	
PAIN PERCEPTION ASSESSMENT	X	X	X	X	X	
ELIGIBILITY CRITERIA	X					
AE ASSESSMENT	X	X	X	X	X	X
IP ASSIGNMENT	X					
ICO TEST	X			X	X	
DELIVERY OF THE IP	X					
START OF INTERVENTION	X					
DELIVERY/TRAINING OF SUBJECT'S DIARY	X					
ADHERENCE ASSESSMENT		X	X	X	X	
RETURN/EVALUATION OF THE SUBJECT'S DIARY					X	
RETURN OF PI					X	

BV, BASELINE VISIT; V1, FOLLOW-UP VISIT 1; V2, FOLLOW-UP VISIT 2; V3, FOLLOW-UP VISIT 3; FV, FINAL VISIT; SC, SAFETY CALL; ICF, INFORMED CONSENT FORM; VA, VISUAL ACUITY; AE, ADVERSE EVENTS; IP, INVESTIGATIONAL PRODUCT; ICO, OCULAR COMFORT INDEX.

Table 2. Study schedule.

2. Introduction and Background

2.1 Theoretical framework

Refractive surgical procedures aim to improve visual acuity, without the aid of over-the-eye lenses or contact lenses, by correcting spherocylindrical errors through reshaping the central cornea. In myopic patients, the curvature is reduced, while hyperopic patients require an increase in the central curvature. [1]

The modern era of refractive surgery began in 1983 with the introduction of the excimer laser to modify the structure of the cornea. [2]

Keratectomy Photorefractive (PRK) was first introduced over 30 years ago [3], this procedure was developed to alter the eye's refractive power in a predictable and permanent manner. [4]

PRK is one of the most popular and effective refractive procedures for correcting refractive errors. It involves reshaping the corneal surface following laser ablation of the stroma, after carefully removing the epithelium. [5] After the procedure, reepithelialization occurs through fibroblast migration and collagen synthesis. [6]

PRK was initially established as a surgical procedure for the refractive correction of myopia; however, its popularity declined in favor of laser-assisted in situ keratomileusis (LASIK). However, PRK is still a technique used in conditions where LASIK is not possible. [7]

PRK is a safe and effective technique for correcting low and moderate myopia. [8]In high myopia, its use is controversial because other factors, such as UV rays, are involved in the development of adverse effects, such as corneal haze, associated with the surgical procedure. [6]

PRK has also been shown to be an effective and safe surgical procedure in the correction of compound myopic astigmatism [9]and hyperopia. [10] [6]

Corneal expenditure or depth of corneal ablation in PRK can be calculated using the Munnerlyn formula ($t=S^2 D/3$), where t is the amount of corneal tissue resected in microns, S is the diameter of the optical zone of ablation in millimeters, and D is the refraction in diopters. For example, if the optical zone of ablation were 6 mm and the diopters to be corrected were -4, the corneal expenditure or depth of corneal ablation would be 48 microns. [3]

PRK is an option for patients with myopia up to -12 diopters, astigmatism up to -6 diopters, and hyperopia up to +5 diopters. [6]Results are better and more predictable at lower levels of each; a high refractive error correlates with a higher likelihood of corneal regression and haze. [6]

Some of the most well-known adverse effects of PRK are corneal haze (cloudiness), corneal reepithelialization abnormalities, and pain. [11] The development of adjuvant [12]mitomycin C (MMC) therapy in PRK as prophylaxis for corneal haze has demonstrated benefits in the refractive correction of high myopia. [13] [14]

In the early days of PRK, surgical optic zone diameters ranged from 4 to 6 mm, with computer algorithms used to control wound depth. Theoretically, corrections of up to -7 diopters removed less than 10% of the axial corneal thickness. [4]

Currently, optical zone diameters of 6.5 mm and 7.0 mm in PRK have proven to be very successful in correcting refractive errors. [15]

One disadvantage of PRK is the postoperative ocular pain associated with corneal de-epithelialization , which leads to the exposure of corneal nerve fibers and the release of inflammatory factors. [16] The pain associated with the PRK procedure improves with corneal re-epithelialization.

In PRK, photoablation reaches the subbasal nerve plexus and anterior corneal stroma, leaving injured nerve fibers at the base and edges of the corneal ablation. [17] Cytokines and nerve growth factor (NGF) released after corneal ablation can also sensitize corneal nerves, lowering the excitation threshold. [18] [19]

Various modifications to the conventional PRK technique have been introduced in an attempt to reduce pain and accelerate epithelial recovery; these attempts have focused primarily on the corneal de-epithelialization method . [20]

Rodrigues et al. reported complete epithelialization in all their patients by day 5 post-PRK; however, they noted that a limitation of their study was that they did not perform daily monitoring of the epithelial healing process. [21]

PRK is not indicated in patients with cataracts, glaucoma, and uncontrolled ocular surface diseases such as blepharitis, dry eye syndrome, and allergies. [6] Keratoconus and other corneal

abnormalities, such as corneal ectasias, thinning, edema, interstitial or neurotrophic keratitis, and extensive vascularization, are considered absolute contraindications. [6]

Patients with active connective tissue diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are considered less than ideal candidates for PRK because of the increased risk of corneal haze . [6]

patient's manifest refraction as well as [6]cycloplegic refraction should be obtained . Pupil size should be measured, as large pupils can contribute to glare and halos postoperatively. Slit-lamp examination is necessary to rule out significant corneal abnormalities such as neovascularization, keratoconus, or the presence of a cataract. Corneal pachymetry allows for the determination of corneal thickness and will detect keratoconus or other types of corneal ectasia. The eyelids and tear film require examination for signs of blepharitis or dry eye. [6]

Preoperative medications are usually administered 20 minutes before refractive surgery. To reduce postoperative pain, nonsteroidal anti-inflammatory drops such as ketorolac tromethamine 0.5% or diclofenac 0.1% can be applied. Topical anesthetic drops such as tetrazine hydrochloride 0.5% or proparacaine 0.5% are placed in the eye a few minutes before surgery. Topical fluoroquinolones such as ciprofloxacin 0.3%, moxifloxacin 0.5%, and gatifloxacin 0.3% are administered to reduce the possibility of infection. Sedation may be considered. [22]

Postoperative treatment for PRK includes topical ophthalmic medications such as nonsteroidal anti-inflammatory drugs, antibiotics, steroids, and lubricants, as well as a soft bandage contact lens. Patients should be informed that their vision will likely be blurred while the corneal surface re-epithelializes. [6]

Corneal reepithelialization is complete in most patients by the 3rd postoperative day. [6]

Long-term visual outcomes for patients undergoing PRK are generally very good. [6] Alio et al. reported in a study of patients with a 15-year follow-up that 55% of eyes were within +/- 1 diopter and 85% within +/- 2 diopters. [23]

When comparing LASIK and PRK, systematic reviews have shown no differences in long-term efficacy, accuracy, or adverse effects in patients with low to moderate myopia [24] [25], however, LASIK has a shorter recovery time and less postoperative pain.

The characteristics of the tear film and the hydrodynamics of the tear system cause ophthalmic formulations to have a short retention period on the ocular surface, so frequent application is necessary to achieve the appropriate concentration of the substance used. [26]

Pharmacokinetic studies conducted in 12 patients with dry eye showed that 0.2% sodium hyaluronate (SH) solution had a mean residence time on the ocular surface of 11.1 minutes, while for 0.3% SH solution it was 23.5 minutes, these solutions were eliminated from the ocular surface in approximately 45 minutes through the tear system. [27]

Benzalkonium chloride is the most widely used preservative in multi-dose ophthalmic products. [28] It is a bactericidal and antifungal agent that helps minimize the growth of microorganisms in multi-dose containers. [28] However, there is scientific evidence supported by *in vitro, in vivo,* and clinical models of the disruptive behavior of benzalkonium chloride on tear stability. It also causes cellular damage to the corneal and conjunctival epithelium, induces inflammatory changes, and leads to a decrease in the function of the corneal epithelial barrier. [29] [30]

With the development of new technologies, such as the Novelia® bottle used in Lagricel® Ofteno multidose , a new generation of preservative-free products in multidose presentations has been created at an affordable price. The Novelia® bottle 's Preservative-Free system has proven capable of protecting the contents from contamination, adding safety to the drug's use by avoiding the addition of preservatives. [31] [32]

In 2019, Laboratorios Sophia SA de CV conducted a Phase I clinical trial to evaluate the safety and tolerability of Lagricel® Ofteno multi-dose ophthalmic solution compared to Lagricel® Ofteno single-dose. A total of 34 healthy subjects were evaluated in two parallel groups, demonstrating that both products are safe and tolerable. [33]

This study will be conducted in a population diagnosed on the 1st day post-surgical with PRK, and will evaluate the efficacy of Lagricel ® Ofteno multidose (Sodium Hyaluronate 0.4%) compared to Thealoz ® Duo in corneal reepithelialization after PRK.

2.2 Background on the product under study

2.2.1 Pharmacology of the investigational product

Sodium hyaluronate (SH) is a glycosaminoglycan with viscoelastic rheology. It is composed of repeating units of N-acetyl-D-glucosamine and sodium-D- glucuronate . [34] SH has a high water-retention capacity, which is one of its reasons for being present in a large number of artificial tear formulations, as it improves ocular hydration, reduces surface friction, and has excellent biocompatibility. [35] [36]

Hyaluronic acid-derived SH shares many of its beneficial properties with hyaluronic acid. In addition to retaining water, which makes it a good humectant and lubricant, SH is hypoosmolar. This latter characteristic allows it to decrease the osmolarity of tears and mucinoid filaments . [37]

2.2.2 Product efficacy under investigation

The efficacy of 0.15% SH in the treatment of patients with dry eye syndrome has been determined, using 1 drop every 4 hours in each eye for 30 days. It was shown that this pharmacological agent decreases inflammatory markers expressed by cells of the corneal surface (macrophages and T lymphocytes) and restores the tear film, which results in a decrease in corneal inflammation. [38]It can be postulated that by decreasing inflammation of the ocular surface with SH, there would be a greater density of goblet cells in the conjunctiva and less apoptosis in corneal cells. [35]

In another clinical trial, the efficacy of 0.18% SH in the treatment of dry eye was evaluated and shown to be more effective than 1% carboxymethylcellulose . Using fluorescein staining as a marker of corneal status, it was determined that SH restores the corneal surface more quickly and reduces dry eye symptoms. [39]

Hyo L et al., evaluated the efficacy of a hypotonic formulation of 0.18% SH in the treatment of dry eye. The study included 30 patients with mild dry eye syndrome, divided into: Group 1: Consisting of 15 patients who received isotonic preservative-free 0.1% SH (300 mOsm / l) 1 drop 4 times a day for 90 days, Group 2: Consisting of 15 patients who received hypotonic preservative-free 0.18% SH (150 mOsm / l) 1 drop 4 times a day for 90 days. The study included 30 patients with moderate dry eye syndrome, divided into: Group 3: 15 patients exposed to isotonic 0.1% SH (1 drop 4 times a day) + fluorometholone 0.1% (2 times a day) + cyclosporine 0.05% (2 times a day). Group 4: 15 patients

exposed to hypotonic 0.18% SH (1 drop 4 times daily) + fluorometholone 0.1% (twice daily) + cyclosporine 0.05% (twice daily). The results demonstrated that the use of 0.1% and 0.18% SH were equally effective in treating mild to moderate dry eye syndrome after 90 days of intervention, considering the stability of the tear film and improvement of the corneal surface. [40]

In a phase II clinical study, the efficacy and safety of Lagricel® Ofteno was evaluated compared to 0.2% polyacrylic acid gel (Viscotears®) after LASIK surgery. This was a prospective, randomized, controlled study that included 30 patients who received the study drug three times a day (TID) for 28 days. Outcome variables included: red eye, foreign body sensation, dryness sensation, pain, photophobia, BCVA (best corrected visual acuity), conjunctival hyperemia, corneal surface integrity, corneal opacity, rose bengal staining, and periocular and fundus findings. The efficacy of Lagricel® Ofteno was demonstrated in this study. [41]

2.2.3 Safety of the investigational product

The safety of Lagricel® Ofteno has been evaluated in several clinical studies. A Phase II clinical study evaluated its safety and efficacy compared to 0.2% polyacrylic acid gel (Viscotears®) after LASIK surgery. This study demonstrated the safety of Lagricel® Ofteno. [41]

In another randomized, controlled, crossover clinical study in 20 patients, the efficacy in reducing signs and symptoms (e.g., red eye, foreign body sensation, and dryness) associated with dry eye disease was tested. [42]

A phase IV clinical study was recently completed evaluating the effect of bromfenac 0.09% ophthalmic solution on conjunctival hyperemia in patients with pterygium, administered twice daily, compared with placebo. Both groups concomitantly used Lagricel® Ofteno TID. The results demonstrated the efficacy of both arms in reducing hyperemia and symptoms associated with pterygium, with statistical significance when compared to baseline. In turn, the bromfenac group was statistically superior to the placebo group. No AEs related to Lagricel® Ofteno were reported. [43]

2.2.4 Summary of the pharmaceutical development of the investigational product

Lagricel® Ofteno multidose has been developed by Laboratorios Sophia, SA de CV. It has undergone physicochemical characterization and a protocol for accelerated and long-term stability. The formulation is identical to that of Lagricel® Ofteno single-dose in a multidose presentation.

2.3 Background on the research

2.3.1 From the research question

There is no prior documented information on the efficacy of Lagricel® Ofteno multidose in corneal reepithelialization following PRK. However, the efficacy and tolerability of Lagricel® Ofteno single-dose has been previously evaluated in two single-center clinical studies . [41, 43, 42, 43] The efficacy and tolerability of Lagricel® Ofteno multidose have been evaluated in a phase I study.

In the three clinical studies of Lagricel® Ofteno single-dose and Lagricel® Ofteno multidose, the outcome variables evaluated were TRPL, comfort, blurred vision, ocular surface staining, and the incidence of AEs. Both formulations were well tolerated, and the final results were statistically significant when compared with baseline.

2.4 Risk benefit assessment

2.4.1 Known potential risks

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

The only anticipated side effects with ophthalmic applications are burning, a foreign body sensation, and blurred vision. These are mild and transient, lasting no more than one minute post-instillation. [33, 41, 42, 43]

2.4.2 Known potential benefits

As these subjects were diagnosed with post-PRK surgery, they are expected to show improvement by reducing the time to complete corneal reepithelialization after surgery and, secondary to this, also improving ocular comfort during this study.

2.5 Problem statement

Although corneal de-epithelialization is part of the standard PRK procedure, shortening the epithelial regeneration time is an important part of the post-surgical recovery process, which is why the initial treatment common denominator is the use of ocular lubricants. There are a wide variety of topical lubricants; however, there is no evidence that one is better than another.

Although ocular lubricants have not been shown to be sufficient to completely resolve the alteration of the ocular surface secondary to corneal de-epithelialization observed in post-PRK surgery patients, they have been shown to be effective in providing protection to the ocular surface and reducing symptoms and clinical findings.

Lagricel® Ofteno multidose, obtains its viscoelastic, hypo-osmolar and water retention properties from SH, to function as an effective lubricant that protects the ocular surface, reconstitutes the tear film and contributes to corneal reepithelialization without the association of a preservative.

2.5.1 Justification

Patients undergoing post-PRK surgery will need to use ocular lubricants to reduce the symptoms associated with corneal de-epithelialization. Ocular lubricants are the first line of treatment for ocular symptoms related to corneal de-epithelialization secondary to PRK. [44] Preservative-free lubricants have gained importance by reducing the deleterious effect of the accumulated dose of preservatives. One of the disadvantages of preservative-free ophthalmic products is their single-dose presentation, which increases treatment costs for various reasons, including product loss.

With the development of new technologies, a new generation of preservative-free products in multi-dose presentations has been created. Lagricel® Ofteno multidose is a preservative-free lubricant in multidose presentation for which documentation of its efficacy in corneal reepithelialization after PRK compared to Thealoz ® Duo is required .

3. Objectives and hypotheses

3.1 Main objective

- To evaluate the efficacy of Lagricel® Ofteno PF compared to Thealoz ® Duo in corneal reepithelialization of post-PRK surgery patients.

3.2 Specific objectives

- To evaluate the safety of Lagricel® Ofteno PF compared to Thealoz® Duo by the incidence of unexpected adverse events (AEs) in post-PRK surgery patients .
- To determine the efficacy of the multidose Lagricel® Ofteno formulation compared to Thealoz® Duo in corneal reepithelialization of post-PRK surgery patients by means of photographic recording using fluorescein.
- To determine the efficacy of the multidose Lagricel® Ofteno formulation compared to Thealoz® Duo in corneal reepithelialization of post-PRK surgery patients using the ICO test .
- To determine the efficacy of the multidose Lagricel® Ofteno formulation compared to Thealoz® Duo in corneal reepithelialization of post-PRK surgery patients by assessing pain perception.
- To determine the safety of the multidose Lagricel® Ofteno formulation compared to Thealoz® Duo in corneal reepithelialization of post-PRK surgery patients through changes in visual acuity.

3.3 Hypothesis

H_0: Lagricel® Ofteno PF does not contribute to complete corneal reepithelialization at the 3rd day of post-PRK treatment and is not equivalent to Thealoz ® Duo .

$$H_0: |\mu A - \mu B| \geq \delta$$

H_1: Lagricel® Ofteno PF contributes to complete corneal reepithelialization on the 3rd day of post-PRK treatment and is equivalent to Thealoz ® Duo .

$$H_1: |\mu A - \mu B| < \delta$$

4. Study design

4.1 Design Overview

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

4.2 Justification of the study design

The study design (clinical trial) is considered the highest standard of data quality when exploring the effect of an intervention. The drug development phase (Phase IV) corresponds to the study objective, which is to evaluate the post-marketing efficacy and safety of products. The presence of parallel groups allows for comparisons between the intervention groups on outcome variables. Due to the nature of the design, blinding was considered for this study.

4.3 Expected duration

The total duration of the study, from the first patient visit to the final report, is estimated to be 11 months.

The approximate duration of each subject in the study is up to 18 days.

5. Study population

5.1 Eligibility criteria

5.1.1 Inclusion criteria

- Be between 18 and 45 years old.
- 1-day post-PRK surgery period (right eye will be evaluated for study).
- Having performed the epithelium removal technique with 20% alcohol (ethanol) for a time within the range of 30-40 seconds.
- Having used a de-epithelialization area (transition area) in the range of 8-9 mm.
- Surgical corneal ablation surface from 5.5 mm to 6.0 mm.
- Corneal expenditure less than or equal to 60 microns.
- Pre-surgical refraction of -1.0 to -4.5 D of myopia or myopic astigmatism (the sum of both values in this case, spherical equivalent no greater than -4.5).
- Have the ability to voluntarily grant signed informed consent.
- Be able and willing to comply with scheduled visits, treatment plan, and other study procedures.
- Be willing to modify your lifestyle activities. See 5.3 Lifestyle considerations
- Women of childbearing potential must ensure continued use (started ≥ 30 days prior to signing the Informed Consent Form [ICF]) of a hormonal contraceptive method or intrauterine device (IUD) during the study period.

5.2 Criteria for exclusion and substitution of subjects

5.2.1 Exclusion criteria

- Transurgical complications and during the post-surgical period prior to inclusion in the study.
- Use of mitomycin during PRK.
- PRK retreatments or history of other types of refractive surgery.

- For women: be pregnant, breastfeeding, or planning to become pregnant during the study period.
- Having participated in another clinical research study ≤ 30 days prior to the screening visit.
- Having previously participated in this study.
- Present an additional diagnosis of:
- Allergic, viral or bacterial conjunctivitis.
- Dry eye.
- Anterior blepharitis.
- Parasitic eye infections (e.g., Demodex).
- History of ocular herpes.
- History of ocular inflammatory processes (such as uveitis).
- History of corneal or conjunctival ulcers.
- Glaucoma.
- Have a history of drug addiction or dependence, currently or within the last two years prior to signing the ICF.
- Have a history of ocular surgical procedures within the last 3 months prior to signing the ICF.
- Having another medical condition, acute or chronic (such as Diabetes Mellitus type I / II, autoimmune diseases or HIV) that, in the opinion of the investigator, could increase the risk associated with participation in the study or the administration of the investigational product, or that could interfere with the interpretation of the study results.
- Use medications (such as retinoic acid) that, in the opinion of the investigator, 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 study results.
- Have a known hypersensitivity to the components of the investigational products.
- Be or have an immediate family member (e.g., spouse, parent/legal guardian, sibling, or child) who is an employee of the research site or the sponsor and who is directly involved in this study.

5.2.2 Subject substitution

The sponsor may decide to replace subjects who withdraw their ICF or those who are lost to follow-up, if it is necessary to balance the study groups so that they are evaluable.

5.3 Lifestyle considerations

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

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

5.4 Scrutiny failures

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

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

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

Subjects who do not meet the eligibility criteria for participation in the study due to a specific modifiable factor may be re-screened. Subjects in this situation must use the same initial screening number.

5.5 Recruitment and retention strategies

This is a Phase IV study, planned to be conducted in at least four centers. The selected centers will be responsible for subject recruitment.

The minimum expected recruitment rate is 1 subject every 3 days.

The duration of the subject's participation in the study is approximately 18 days, during which they will only be required to attend four visits after the initial visit. Subjects will be eligible for travel and visit allowances. Other strategies to improve subject retention include:

- Clearly communicate the importance of the study and the benefits that the population will obtain from its results .
- Make calls or send text messages to remind yourself of appointments or activities to do.
- Provide a printed calendar and an identification card to remind you of appointments and activities to be carried out, as well as their estimated duration .
- Offer flexible business hours.
- Systematic organization of the study procedures, so that the subject does not stay longer than necessary during his visit.
- Minimize subject wait times.

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

5.6 Procedure in case of loss of follow-up

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

If the participating subject does not attend their appointment, the research site will call to determine the reason and attempt to schedule a new appointment within the established window or an unscheduled appointment. If an appointment cannot be scheduled, the subject will be asked about the presence of adverse events and the reason for discontinuing the study, as minimum information.

A loss to follow-up <20% is not considered to represent a problem for the validity of the results obtained. [45, 46]

5.7 Subject identification

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

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

Example:

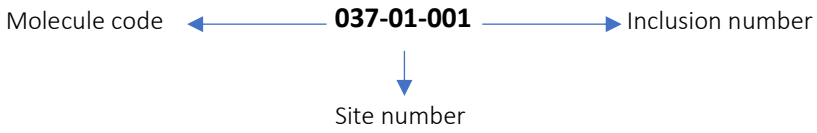
A. A rieh Daniel M ercado C arrizalez
   a. Initials: AMC

B. J uan D e la Torre O rozco
   b. Initials: JDO

During the screening phase (during the initial visit), you will be assigned a consecutive participant number, using three consecutive digits. Once the subject has been selected, they will be assigned a number that will be used to identify them throughout the study. This code will consist of eight numbers in the following order from left to right:

- Three digits of the molecule under study according to the sponsor's designation.
- two digits corresponding to the research center number.
- three digits of the consecutive number assigned to its inclusion in the research center.

Example:



6. Investigational product

6.1 Managed products

6.1.1 Investigational product

- Generic name: Sodium hyaluronate.
- Distinctive name: Lagricel® Ofteno multidose.
- Active ingredients: Sodium hyaluronate 0.4%.
- Excipients: sodium chloride, monobasic sodium phosphate monohydrate, anhydrous dibasic sodium phosphate, water for the manufacture of injectables.
- Pharmaceutical form: ophthalmic solution.
- Presentation: multi-dose dropper bottle.
- Prepared by: Laboratorios Sophia S.A. de C.V.
- Solution description: Clear solution, free of visible particles.
- Packaging description: White bottle with a capacity of 10 mL, made of low-density polyethylene.

6.1.2 Reference product

- Generic name:Trehalosa 3% / Hialuronato de sodio 0.15%.
- Distinctive name: Thealoz ® Duo .
- Active ingredients:Trehalosa 3% / Hialuronato de sodio 0.15%.
- Excipients: sodium chloride, trometamol , hydrochloric acid, water for the manufacture of injectables.
- Pharmaceutical form: Ophthalmic eye drop solution.
- Presentation: multi-dose dropper bottle.
- Prepared by:Laboratorios THEA.
- Solution description: Clear solution, free of visible particles.
- Packaging description: white bottle with 10 mL capacity.

6.1.3 Dose of the investigational product

The same dosage will be used for Lagricel® Ofteno multidose presentation and Thealoz ® Duo :

- One drop 4 times a day, OU, for 14 days (minimum time between applications of 3 hours).

6.1.3.1 Justification of the dose

Ophthalmic lubricants are generally prescribed as needed (*pro re nata* [PRN]). [47]However, in the Asbell study *et al.* compared the efficacy of four times daily (QID) dosing versus PRN dosing. Their results showed that, although there was no difference in clinical signs, the QID group had greater symptom improvement. [48]For this reason, they propose the four-times-daily dosing regimen.

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

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

Reception will be carried out by the assigned research team staff. The primary packaging (box) must be inspected for its condition. If it shows alterations or defects in its integrity that, in your opinion, could have damaged the contents, you must report this to the sponsor. If the package shows no significant defects, it will be opened.

Inside, you should find the receipt acknowledgment document and the temperature *data logger* . You must check that the recorded temperature meets the specifications for its transport and storage. The contents (PI) will be verified with what is reported on the document. If the document matches the contents, the receipt will be signed and sent to the sponsor. If not, the sponsor will be notified.

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

Storage temperature should be no more than 30°C.

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

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

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

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

6.3 Concomitant treatments and medications not authorized during the study

The use of concomitant medications by any route of administration will not be permitted during the intervention period, except for those specified for the study procedures. The purpose of this restriction is to avoid drug interactions that could alter the results of the evaluated variables .

Permitted medications:

- Ophthalmics: Tetracaine 0.5% (Ponti), Tropicamide 0.8% / Phenylephrine 5% (TP) (for study procedures), PI, Ciprofloxacin 0.3% (Sophixin), Fluorometholone 0.1% (Flumetol NF).
- No ophthalmic: Contraceptives hormonal, painkillers: Stadium (Dexketoprofen , oral 25 mg/6hrs: initial intake after surgery, after PRN [For Necessary Reason]) and Supradol (Ketorolac, sublingual 30 mg every 6 hours: take initial later to surgery, after PRN). TO criterion of the researcher HE They may use systemic medications whose effects do not modify the parameters of efficacy or safety of this protocol, but they must be notified to the committee scientist of the sponsor for determine the situation of the participant according to corresponds.

Medications prohibited:

- Any medicine ophthalmic that No HE find in the list of medications allowed. Medications systemic: immunomodulators and tetracyclines.

6.4 Procedure for monitoring and measuring adherence

For more than four decades, numerous studies have been conducted on the appropriate way to measure and quantify medication adherence; however, none have reached a consensus that could serve as the gold standard, whether in cross-sectional or longitudinal studies. [49, 50, 51, 52, 53, 54, 55, 56]

There are different procedures for measuring adherence to pharmacological interventions. The most common procedure involves self-reports , which include patient interviews, questionnaires, and self-monitoring diaries. Their strengths are speed, flexibility, low cost, and ease of implementation; they have a high degree of specificity for nonadherence, but their sensitivity and reliability for adherence are low. [56, 57]

Biochemical measurement of the drug, or its metabolite, is one of the methods that best confirms drug use. However, in addition to being costly and impractical, it is of little use in ophthalmic applications, as peripheral concentrations may be undetectable; and samples from other tissues require more invasive methods that would be inadvisable. [56]

Medication counting is another way to measure adherence. Classically referred to as "pill counting," in ophthalmology it is translated as the weight of the bottle. This is a simple, inexpensive, and noninvasive method. The main disadvantages of this method are: 1. It cannot confirm the application of the medication (it could have been intentionally dropped or instilled outside the eye), and 2. It depends on the subject bringing the medication back. [56, 57]However, in this study, the initial and final weight of the bottle will also be taken into account to measure adherence.

A multi-method approach to adherence measurement is recommended. Because there is no ideal adherence measure, it is appropriate to use more than one method when aiming to achieve results that resemble reality. Selecting two or more methods allows for balancing their strengths and weaknesses, thus more accurately capturing adherence levels. [55]

Adhesion assessment will be based on the bottle's weight and will be performed as follows: drop weight, initial container weight, final container weight, and a calculation of the total number of applications. The following simplified formula will be used:

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

Where:

Ad = adhesion

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

P_f = weight of the container returned by the subject

P_T = weight of the dosage indicated for the intervention

$$P_T = (P_g)G$$

Where:

P_g = weight of a drop of the intervention, determined by the research and development department

G = number of applications indicated for the intervention

Packaging that does not maintain its physical integrity will not be considered for the calculation of adhesion.

In cases where the container is not returned, or has not maintained its physical integrity, adherence will be measured using the subject's diary, as follows:

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

Ad = Adhesion

A_r = Registered applications

A_i = Indicated applications for investigational products

There is no standardized parameter for defining adequate adherence; it must be defined and outlined by the objectives of the particular research. [56]

For this study, a minimum adherence of 70% will be considered necessary to meet the research objectives, both through the subject diary and by bottle weight. Therefore, subjects with less than 70% adherence will not be considered for the efficacy analysis; they will only be included in the safety analysis.

6.5 Strategies to improve adherence

1. The Principal Investigator (PI) will educate the subject on the importance of correctly applying the research product (PI) to achieve the study objectives.
2. Direct questioning by the IP regarding the application of the PI.

3. Delivery of a printed calendar specifying the date of the visit and its activities.
4. Training in completing and reviewing the “Subject Diary.”
5. If deemed necessary, text messages may be sent as reminders. The content of these messages must be approved in advance by the Research Ethics Committee (REC).

7. Methods and procedures of the study

7.1 From the research center

This study will be conducted at research centers previously evaluated by the sponsor. These centers will be institutions or establishments where health research is conducted in compliance with current regulations.

The number of centers to be included will be determined based on the capacity of the centers evaluated during the feasibility studies. The suggested number is four or more.

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

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

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

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

7.2 Clinical study registration

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

7.3 Randomization

Subject randomization will be performed using a computerized allocation system. After signing the ICF, the patient will receive a patient number, which will be used to coded all information pseudo - anonymously during data collection and completely anonymously during analysis.

The generation will be carried out by a third party authorized by Laboratorios Sophia, SA de CV, through its electronic system. The information corresponding to this third party will be found in the file.

Although this is an open-label study, secondary packaging will be blinded.

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

- Name, address and telephone number of the sponsor.
- Pharmaceutical form and route of administration.
- Batch number.
- Legend “Exclusively for clinical studies”
- Expiration date.

7.4 Outcome variables

7.4.1 Primary efficacy outcome variable

- Corneal reepithelialization time (TEAE: day 1, 2, 3 and 7).

7.4.2 Primary safety outcome variable

- Incidence of AE (TEAE: day 2, 3, 7 and 15).
- Changes in VA (TEAE: day 2, 3, 7 and 15).

7.4.3 efficacy outcome variables

- Percentage of postoperative corneal reepithelialization (TEAE: day 1, 2, 3 and 7).
- ICO test score (TEAE: day 7 and 15).
- Pain perception assessment (TEAE: day 2, 3, 7 and 15).

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

Variable	Type	Unit	Measurement Method	Normal Value	Evaluation Time	Statistical Test
Primary efficacy outcome						
Corneal reepithelialization time	Discrete	Days	Direct observation and photographic record	3 days	BV, V1, V2, V3	Student's t-test / Mann–Whitney U*
Secondary efficacy outcomes						
Percentage of daily post-surgical reepithelialization	Continuous	Percentage (%)	Direct observation	100%	BV, V1, V2, V3	Student's t-test / Mann–Whitney U*
ICO Score	Discrete	Numeric (score)	Questionnaire	0	BV, V3 FV	Student's t-test / Mann–Whitney U*
Pain perception	Ordinal	Severity and frequency scales	Questionnaire	Absent / None	BV, V1, V2, V3, FV	χ^2 (Chi-square) or Fisher's exact test; McNemar test *
Primary safety outcome						
AE (number)	Discrete	Number of cases (n)	Counting	0	BV, V1-3, FV	Student's t test
AE (ocurrance)	Nominal categorical	Present / absent (-)	Observation	Absent	BV, V1-3 and FV	χ^2 (Chi-square) Pearson or Fisher exact.
VA	Discreet	Fraction (-)	Snellen chart	1	BV, V1-3 and FV	Student's t-test / Mann–Whitney U*

Abbreviations: ICO = Eye Comfort Index; AE = Adverse Event; VA = Visual Acuity.
*Applied when the assumption of normality is not met.

Table 3. Operational definition of variables

The variables, method, and scales used to measure them are described in detail below. They are listed in order according to Table 3.

7.4.4.1 Corneal reepithelialization

Corneal reepithelialization will be assessed by photographic record using fluorescein. The bandage lens will be removed, then a drop of topical anesthetic will be instilled in the conjunctival fornix. A second drop will then be applied to the tip of the fluorescein strip, allowing it to sit on the strip for 5 seconds to elute the dye, finally shaking off the excess. A small contact of the strip with the conjunctiva is made in the temporal fornix while the patient looks upward, without damaging the conjunctiva. A photographic record will be taken to evaluate the progress of the reepithelialization process. The magnification or magnification used in the slit lamp for the photographs will be 6x and 16x. A minimum of 3 frontal photographs will be taken, visualizing the entire corneal surface by magnification, 6 photographs in total per patient per visit. The photographic evidence will be stored in a virtual storage network and the images will be recorded in accordance with the stipulations of the current operating manual. After taking photographs, a new contact lens will be placed.

The IP will record in the file and the eCRF the daily percentage of progress of the corneal reepithelialization process (considering the corneal de -epithelialization zone a total of 100%) on the post-surgical days indicated in the protocol.

Management as AE: Corneal stains that do not show 100% progress in the corneal reepithelialization process within the first 3 days postoperatively will be considered AE due to lack of efficacy.

7.4.4.2 ICO Score

The OCI is a questionnaire designed to measure ocular surface irritation using Rasch analysis to produce estimates on a linear interval scale (scores: 0–100). Similar to the index for ocular surface diseases, the Ocular Comfort Index (OCI) assesses symptoms. The OCI contains items that focus on discomfort associated with ocular surface disorders. Each of these questions has two parts, which separately inquire about the frequency and severity of symptoms. [58]

The evaluator will give the questionnaire to the subject and allow them to answer it calmly without any pressure or coercion. They will only assist them if they have difficulty understanding any of the questions.

Management as an AE: The ICO assesses symptoms, which may or may not be related to an AE. The ICO score alone should not be considered an AE.

7.4.4.3 Pain perception assessment

The subject will be questioned directly about the presence of pain. They will respond to the severity and frequency of symptoms, such as:

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

Frequency: All the time (4), almost all the time (3), 50% of the time (2), almost never (1), never (0).

The number corresponding to each symptom will be recorded in the eCRF .

7.4.4.4 Adverse events

As defined in section 8.1.1, an EA It is any unfavorable medical occurrence in a subject to whom a PI is administered, regardless of the causal attribution.

The management of AEs will be carried out as described in the Adverse Events section.

any AEs that the study subjects present in the corresponding section of the eCRF and will also report them in the clinical record.

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

The expected adverse effects associated with the use of PIs are: blurred vision, burning, eye irritation, foreign body sensation, and sticky eyelashes. They are expected to be transient, lasting no more than 30 seconds after PI instillation , and to be mild in intensity.

7.4.4.5 Visual acuity

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

Snellen notation is described as the distance at which the test is performed divided by the distance at which the letter vertically equals 5 minutes of arc. Thus, at 6 meters, a letter 6/6 (20/20) equals 5 minutes of arc, a letter 6/12 (20/40) equals 10 minutes, and a letter 6/60 (20/200) equals 50 minutes. The Snellen fraction can also be expressed as a decimal (i.e., 20/20 = 1 and 20/40 = 0.5). [59]

VA will be assessed using the Snellen chart. This chart will be placed in a location with adequate natural or artificial lighting and at a distance of 3 m from the subject being assessed. Visual acuity will be measured in each eye, starting with the right eye (RE), asking the subject to keep both eyes open and using an occluder to cover the left eye (LE); the subject will read aloud the lines indicated by the evaluator. The line with the smallest letters visible will be recorded by the evaluator as a fraction of the RE VA in the clinical record. The LE is then assessed using the same method.

Management as an AE: A decrease of more than 2 lines on the Snellen chart, taking into account the baseline visit, should be reported and managed as an AE.

7.4.5 Program of visits and activities of the study

7.4.5.1 Description of activities per visit

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

7.4.5.2 Baseline Visit

- Signature of the ICF: refers to the signing of the written informed consent document. Without informed consent, none of the study procedures can be performed.
- Medical history: It must be carried out in accordance with the provisions of NOM-004-SSA3-2012 of the clinical record. [60]Considering that the clinical record is the unique set of information and personal data of a patient, which is integrated within all types of

establishments for medical care, whether public, social or private, which consists of written, graphic, imaging, electronic, magnetic, electromagnetic, optical, magneto-optical and any other type of documents, in which health personnel must make records, annotations, where appropriate, certificates and certifications corresponding to their intervention in the medical care of the patient, in accordance with the applicable legal provisions.

And it will consist of:

- Medical history: It must be prepared by medical staff and other health professionals, according to the specific information needs of each of them in particular, and must have, in the order indicated, the following sections:
- Interview. - It should include, at a minimum, the following: identification form, if applicable, ethnic group, family history, medical and non-medical history, current condition (inquire about previous conventional, alternative, and traditional treatments), and a history of equipment and systems (especially ophthalmologic).
- Physical examination. - At a minimum, the following should be included: vital signs (temperature, blood pressure, heart rate, and respiratory rate), weight, and height (when applicable).
- Previous and current results of laboratory, office and other studies (when applicable).
- Diagnoses or clinical problems (when applicable).
- Forecast (when applicable).
- Therapeutic indication (when applicable).

- Evaluation of concomitant medications: refers to the IP questioning the subject, inquiring about the use of medications.
- Urine pregnancy test: This refers to the performance of a rapid pregnancy test in all women of childbearing age who wish to enter the study. By childbearing age, we mean women who have experienced menarche but have not yet experienced menopause. Menopause is defined as 12 months since the last menstrual period in women over 40 years of age, or those who have undergone a hysterectomy or bilateral oophorectomy. Women of childbearing age using contraceptive methods, including bilateral tubal obstruction , must undergo a pregnancy test. This test will be performed by the PI or a designated team member according to the instructions on the device provided by the sponsor.

- Vital signs: This refers to heart rate, respiratory rate, systemic blood pressure, and temperature. This information should be recorded in the patient's medical history and progress notes (values outside the range will be reported as AEs at the discretion of the PI).
- VA: See 7.4.4.5
- ICO: See 7.4.4.2
- Corneal reepithelialization : See 7.4.4.1
- Pain perception: See 7.4.4.3
- Ophthalmological evaluation: Refers to the evaluation of the subject's ophthalmic structures, eyelids and adnexa, ocular surface, anterior segment, and posterior segment; not considered within the outcome variables. This evaluation is intended to identify alterations that may interfere with the course of the investigation or identify adverse events. This examination will be recorded in the clinical record; only those considered adverse events will be reported in the CRF.
- Eligibility criteria: Refers to the PI's review, which verifies that the subject can be included in the study if they meet the inclusion criteria and do not meet the exclusion criteria. See 5.1.
- PI Allocation: This refers to determining the intervention the patient will undergo during the study. This assignment will be carried out in accordance with Section 7.3. This assignment will be made at the initial visit (Day 1).
- Delivery of the PI and start of intervention: Refers to the delivery of the PI to the study patient by the research center.
- EA Assessment: See 7.4.4.47.4.4.4
- Delivery of patient materials and instructions for completion: Refers to the IP providing the subject with the subject's diary, identification card, and adherence-enhancing calendar. The assigned staff will provide the subject with pre-training on how to complete the diary.

7.4.5.3 Follow-up visits 1 to 3

- Evaluation of concomitant medications: See 7.4.5.2
- Vital signs: See 7.4.5.2
- VA: See 7.4.5.2
- ICO: Ver 7.4.5.2(only on visit 3)
- Corneal reepithelialization: See 7.4.5.2

- Pain perception: See 7.4.5.2
- Ophthalmological evaluation: See 7.4.5.2
- EA Assessment: See 7.4.5.2

7.4.5.4 Final visit

- Evaluation of concomitant medications: See 7.4.5.2
- Urine pregnancy test: See 7.4.5.2
- Vital signs: See 7.4.5.2
- VA: See 7.4.5.2
- ICO: See 7.4.5.2
- Corneal reepithelialization: See 7.4.5.2
- Pain perception: See 7.4.5.2
- Ophthalmological evaluation: See 7.4.5.2
- EA Assessment: See 7.4.5.2
- Adherence assessment: See 6.4
- Subject Diary Assessment: This refers to the review of the Subject Diary instrument by the PI or designated facility staff; it will be reviewed for correct completion, as well as the patient's comments. The PI may question these comments for AEs. At the PI's discretion, the comments may or may not be reported as AEs.
- Adherence assessment: See 6.4
- Return of PI and Subject Diary: Refers to the return by the subject of the PI and subject diary to the research center.

7.4.5.5 Unscheduled follow-up visits

At the request of the patient or any other individual involved in the study, unscheduled follow-up visits may be conducted to report adverse events. During these visits, all relevant data on reported adverse events must be collected, and an appropriate management plan must be established, if applicable.

7.4.6 Data collection

7.4.6.1 Source documents

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

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

7.4.6.2 Electronic forms of data collection

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

7.4.6.3 Archive

The data collected in this database is anonymous (only the patient number is stored, along with other relevant information). The software used for data capture and storage meets the traceability requirements necessary for conducting clinical studies. The collected data will be stored by the sponsor or designated clinical research organization for a period of 10 years. The master file will remain at the participating institutions under the care of the PI or their team and must be safeguarded for at least 5 years.

8. Evaluation and management of adverse events

8.1 Regulation and standards on adverse events

The registration and reporting of adverse events will be carried out in accordance with the guidelines established in NOM-240-SSA1-2012 Installation and operation of technovigilance and which are in accordance with the international ICH E6 guidelines. [61]

8.1.1 Definition of adverse event

According to the ICH, an AE is any adverse medical occurrence in a patient undergoing clinical research who is administered a pharmaceutical product, regardless of causal attribution.

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

Similarly, according to NOM-240-SSA1-2012, an incident is described as: any event related to the use of a medical device, and an adverse incident is described as: any proven event related to the use of a medical device that has conclusive evidence of the causal relationship between the incident and the medical device, and that could be caused by a malfunction or alteration of the characteristics of the medical device and that could cause death or serious deterioration of the user's health. An adverse incident will not be considered an incident derived from abnormal use or a use different from that recommended by the holder of the sanitary registration of the medical device or its legal representative in Mexico. [61]

8.1.2 Definitions relevant to the classification of adverse events

Severity (serious/non-serious), also called seriousness (serious/non-serious). A serious event is defined as any event that: results in death, is life-threatening, requires hospitalization or prolongs hospitalization, causes permanent or significant disability or incapacity, causes alterations or malformations in the newborn, or other medically important conditions (Medically important event or reaction: A clinical manifestation or adverse event that, in the judgment of the physician, may not be immediately life-threatening, result in death, or cause hospitalization, but that could endanger the patient or require medical intervention to avoid the occurrence of any of the criteria listed in the definition of a serious adverse reaction). [61]

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

Causality. This is the relationship assigned between the drug and the adverse event: certainly caused by the drug, there is clear evidence of causality, i.e. the adverse event reappears with the administration of the drug; probably caused by the drug, there is a high suspicion of causality but no direct evidence is available or it is considered unnecessary or dangerous, i.e. the reaction disappears when the drug is discontinued; possibly caused by the drug, there is additional information suggesting that the cause may be due to another drug or disease; unlikely to be caused by the drug, there is a clear explanation for 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 on the adverse event has been obtained, it remains unclassifiable.

8.1.3 Researcher Responsibilities

The investigator is responsible for verifying the AE by conducting a questioning, reviewing the information recorded in the subject's diary, conducting a relevant physical examination, assessing progress, and providing appropriate medical and pharmacological management. The investigator is

also responsible for monitoring the AE until it is resolved or resolved, and the patient is discharged, following the definitions established in national and international regulations.[61] [62] [63]

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

The attention of the AE will be carried out according to the event attention diagram (see Figure 3. Adverse event ***care***).

The final report prepared by the Clinical Team of Laboratorios Sophia, SA de CV's Medical Management Department will include adverse event reports in compliance with current national and international regulations. [62] [61]

If the research subject develops a chronic adverse event during their participation in the study, such as diabetes or high blood pressure, they will be referred to a healthcare professional for chronic treatment. Follow-up and termination of participation will be in accordance with the provisions of NOM-012-SSA3-2012.[64]

8.1.4 Recording of adverse events in the electronic case report form

The EA registry considers:

- Subject identification information such as: subject number, age, sex, and if applicable, specify the eye.
- Information about the causality of the AE, its relationship to the PI, or to another study-related drug, as appropriate.
- Information on important dates:
    - Date on which the EA occurs.
    - Date on which the IP becomes aware of the same.
    - Date of resolution or outcome, as applicable.
- Information on diagnosis and clinical management.

- If a lack of therapeutic response to PIs is detected, include in concomitant medications the therapy used for the pharmacological management of the adverse event.
- Establish the outcome or resolution of the event:
    - Recovered/Solved
    - Recovered/resolved with sequelae
    - Recovering/resolving
    - Not recovered/unresolved
    - Fatal
    - A stranger
- Information about the investigational product or medicinal product or the medicinal product associated with the AE, ADR or SRAM. As applicable, information concerning the generic name, distinctive name or code of the PI and/or investigational medicinal product must be recorded, as appropriate according to the methodological design of the study. This is relevant in the case of blinded studies or those where placebo is used as comparators, since there are circumstances that justify opening the blind to determine whether the adverse event can be attributable to the active agent, the combination of active agents, or to the pharmacologically inert substance(s), such as the vehicle or additives, as appropriate to the phase of clinical research in which the development of the medicinal product is located. It will also be necessary to record data concerning the batch number, manufacturing laboratory, expiration date, dose, route of administration, start and end dates of administration and/or consumption, reason for the prescription; depending on whether it is an investigational product or medication (a protocol in which the patient is currently participating) or a medication that the subject under investigation consumes for the treatment of underlying concomitant diseases or uses to manage some temporary sign or symptom that does not correspond to the natural history of the pathology that motivated their entry into the research protocol.
- Indicate the withdrawal or continuation of the medication, as appropriate. Indicate whether the adverse event disappears upon withdrawal of the PI, investigational medication, or suspected medication (of causing the event). Also indicate whether a dose adjustment is made, if the event changes in intensity or severity, and if the reaction persists. It is important to indicate whether the AE reappears in subjects who are re-exposed to the medication after having been previously discontinued.

- Information regarding concomitant pharmacotherapy. Indicate the generic name, dose, route of administration, start and end dates, and the reason for the prescription, regardless of whether it is in accordance with the prescribing information or the data sheet or if it is used outside of the regulations or as authorized by the local, national, or international regulatory body.
- Information on relevant clinical history. The analysis of the AE considers the information previously described. However, the clinical context in which the adverse event occurs in the participants in the clinical research protocol is of particular interest. Therefore, information about previous conditions, hypersensitivity or allergy symptoms, previous surgical procedures, laboratory tests or imaging examinations the participant has undergone, etc., that the researcher deems appropriate may be mentioned.

8.1.5 Monitoring of adverse events

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

8.1.6 Procedures for a serious adverse event

The EA care process considers the following stages (Figure 3):

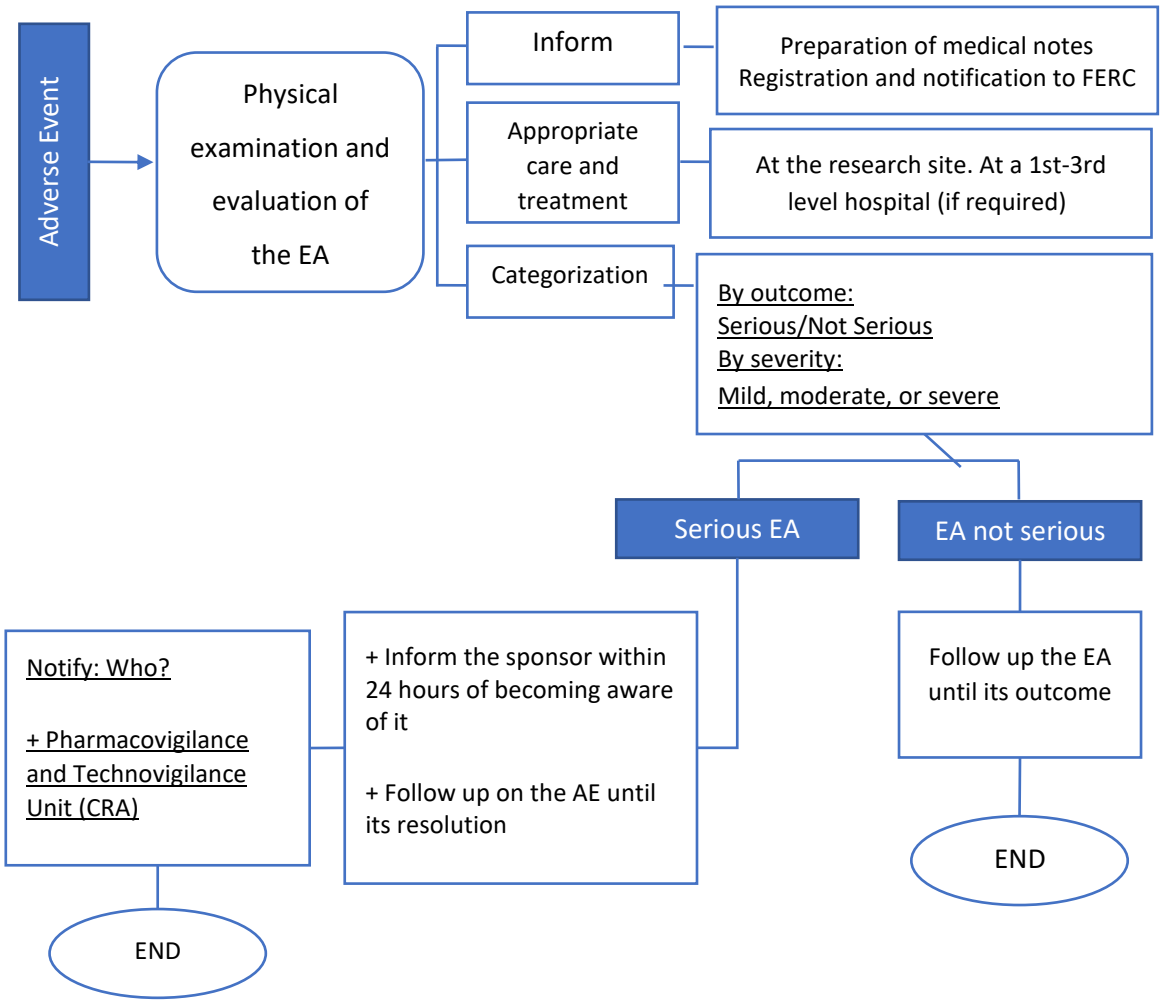


Figure 3. Adverse event care

During the development and conduct of this study, undesirable adverse events or adverse reactions of medical significance may occur in the research subject, which are not necessarily causally related to the investigational medicinal products. These adverse events may occur during the use of investigational medicinal products at doses authorized for human use by a local, national, or international regulatory body. However, it may be suspected that the investigational medicinal product or the investigational medicinal product may cause some undesirable clinical manifestation. AEs, ADRs, or SARs associated with one or more medicinal products may occur during the systematic evaluation of participants (on the days when clinical reviews are scheduled, according to the activity schedule) or suddenly, such that:

1. The researcher should be the first person to whom the patient notifies that he or she has developed or experienced any harmful clinical phenomenon during his or her participation in this study.
2. Based on their clinical judgment, the principal investigator will determine the appropriate treatment for the adverse event/reaction based on the relevant physical examination, history, etc., as well as the analysis of information available in the medical literature and the information contained in the investigator's manual, Prescribing Information, or the comparator drug's data sheet.
3. This care may be provided at the research center or at the hospital with the highest capacity for treatment. Thus, if the patient is referred by the PI to a hospital, they will receive care through a referral system. The referral may be through a card identifying the subject as a study participant and linking them to the pre-established agreement with the institution, or through a referral medical note issued by the PI. Laboratorios Sophia, SA de CV, will pay the costs for medical care for participating patients when the adverse event is associated with or related to the PI or investigational drug.
4. Taking into account the clinical information collected, either during the care provided at the research center or provided by the treating physician(s) at the hospital, the IP will record the AE in his/her clinical note, stating the seriousness, intensity (mild, moderate, or severe), and relationship with the product or drug under investigation.
5. The PI must migrate the relevant data to the eCRF and its respective adverse event section. In the case of serious adverse events, they must be reported to the clinical monitor of the study within 24 hours of becoming aware of them , so that they can then inform the Clinical Team and the UTFLS. Regarding non-serious adverse events, these

will be recorded and appropriately addressed, 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.

Recording the outcome of an AE depends substantially on the PI's follow-up of the subject, as most adverse events (see the investigator's manual) are expected to be ophthalmic in nature; however, systemic changes may occur. Therefore, at the investigator's discretion, the participant's withdrawal or continuation will be considered.

8.1.7 Assessment of causality

Causality assessment is the methodology used to estimate the probability of attributing an observed adverse event to a medication. It considers probabilistic categories according to the available evidence and the quality of the information, based on national pharmacovigilance regulations, the World Health Organization, and the Uppsala Monitoring Center. [61]

The Pharmacovigilance and Technovigilance Unit of Sophia Laboratories (UFTLS) can use the causality assessment provided by the World Health Organization and the Uppsala Monitoring Center as a tool to facilitate the probabilistic categorization of causality. Similarly, the Karch and Lasagna algorithm modified by Naranjo, referred to by Aramendi I, can be used as an aid to perform the causality categorization. In this algorithm, different items are scored, which allow a value to be assigned to the cause-effect relationship between the administration of the drug and the adverse reaction. [64]

No .	Reagent	Score	
		Yes	**No**
1.	There are conclusive previous reports on adverse drug reaction, adverse event or suspected adverse drug reaction	+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 recurred upon administration of the investigational drug/investigational product/investigational drug	+2	-1
5.	There are alternative causes that can provoke this reaction.	-1	+2
6.	The adverse reaction/adverse event/suspected adverse drug reaction occurred after placebo administration	-1	+1
7.	The drug was determined in 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 less 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 with some objective evidence	+1	0
Total score		Total	

Probabilistic category based on the score obtained		
I	The causal relationship is verified	≥ ,9
II	The ADR is likely due to the investigational drug or product	5 to 8
III	The ADR may be due to the investigational drug or product	1 to 4
IV	The causal relationship is doubtful	0

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

Table 4. Karch and Lasagna algorithm modified by Naranjo.

There are no guidelines or algorithms available to determine the causality of an adverse event in the case of the use of a medical device. This means that the relationship is established by the investigator's opinion and the reasoning behind how the adverse event occurred. However, the Pharmacovigilance and Technovigilance Unit of Sophia Laboratories (UFTLS) may use the causality categories described by the Uppsala Monitoring Centre to categorize the probability of an adverse event related to concomitant or experimental treatment. Medical devices, for convenience, may be evaluated as medicines within the categories mentioned in the section "Definitions relevant to the classification of adverse events, Causality."

Thus, the degree of certainty required to establish the PI as the causal agent of the harmful phenomenon occurring to the subject of the clinical study is essential. This can also be indicated directly by the PI based on their clinical experience or through the voluntary application of the aforementioned tool. However, it is important that the researcher and the UFTLS consider the following arguments in favor of a causal relationship:

a) Strength of association, which refers to the number of cases in relation to those exposed.
b) The consistency of the data, that is, the presence of a common characteristic or pattern.
c) The exposure-effect pattern, which determines the relationship with the site of onset, time, dose and reversibility after suppression.
d) Biological plausibility, which refers to the possible pharmacological or pathophysiological mechanisms involved in the development or presentation of the adverse event.
e) Experimental findings, for example, the appearance of anomalous metabolites or high levels of drug or its biotransformation product.
f) Analogy, which refers to the experience acquired with other related drugs, adverse reactions frequently produced by the same family of pharmacological agents.
g) Nature and characteristics of the data, i.e. objectivity, accuracy and validity of the relevant documentation. [65]

9. Study monitoring

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

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

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

9.1 Monitoring of study centers

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

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

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

The closing visit will take place at the end of the study, once the last participant at the site has been discharged from follow-up. During this visit, the monitor will verify that the site has all the necessary documentation for archiving, that all biological samples have been sent for analysis, that all study

drug (used and unused) has been recovered and sent to the sponsor, and that all unused materials have been retrieved.

Details of monitoring are set out in the relevant plan.

9.2 Audit and quality assurance

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

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

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

10. Statistical analysis

10.1 Data analysis

10.1.1 Statistical analysis

Statistical analysis will be performed by staff of Laboratorios Sophia, SA de CV. The statistical package SPSS version 19.0 for Windows (IBM Corporation, Armonk, NY, USA) will be used.

The personnel assigned to statistical data management will be blinded to the intervention groups. Coding will be performed using consecutive numbers for each intervention group.

The data will be collected and organized in an Excel spreadsheet (Microsoft® Office). The data will then be exported to the SPSS platform. Variables will be categorized according to their nature (see Table 3. Operational definition of **variables**).

10.1.2 Data interpretation

Wilk test will be performed to determine whether the distribution is normal in the results obtained in each study group [66]. In case of p <0.05, the Mann-Whitney U statistic will be used for the analysis of quantitative variables or the Wilcoxon rank test when applicable.

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

- Intra-group analysis : Differences within groups will be analyzed using Student's t-statistic . [67]
- Between-group analysis : Differences between groups will be determined using Student's t-test for independent groups.

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.

Statistical analysis to identify significant differences in qualitative variables will be performed by creating pxqy contingency tables and will be carried out as follows:

- Intra-group difference: McNemar's test. This test is applied to p × q contingency tables with a dichotomous trait, with matched subject pairs, to determine whether the row and column marginal frequencies are equal (marginal homogeneity).[68]
- Difference between groups: Pearson's Chi-square test (X^2) or Fisher's exact test for expected values less than 5.

The level of difference to consider significance will be an alpha (α) of 0.05 or less. Both eyes will be considered for the safety report. Only the right eye will be considered for the efficacy report.

For the reporting of adverse events, all participants who were randomly assigned to an intervention group after the baseline visit (intention-to-treat population; ITT) will be considered.

The final results report will be displayed in tables or graphs, as appropriate.

10.1.3 Procedure for handling missing data

- Efficacy: Subjects who meet a minimum adherence of 70% will be included in the statistical analysis to meet the study objective. This is based on the weight of the patient and does not present major deviations from the protocol (PP; per-protocol population). In cases where the container is not returned or has not maintained its physical integrity, adherence will be measured using the subject diary tool.
- Safety: The safety assessment will include in the analysis all subjects (AO) who have been exposed at least once to the intervention, regardless of the visit at which they were eliminated from the study (ITT population).

10.1.4 Deviations from the statistical analysis plan

For the following protocol, a sample size of 96 subjects (48 per group) is suggested. According to the sample size calculation to meet the primary objective of the study, 84 evaluable subjects are required (42 subjects per treatment arm). If this number is not met due to a loss of subjects exceeding the 15% threshold contemplated in this protocol (loss to follow-up or withdrawal from ICF), the sponsor may substitute these subjects to balance the treatment groups.

Results obtained from the replaced subjects will continue to be used for safety analysis and will be part of the ITT population.

10.1.5 Subjects included in the analysis

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

10.2 Sample size calculation

10.2.1 Number of subjects calculated

n = 96 evaluable subjects

48 subjects per arm.

10.2.2 Justification of the sample calculation

The sample size calculation was based on the clinical study by Lee et al. (2001), where they compared the efficacy, safety, and stability of LASEK vs. conventional PRK in mild to moderate myopia. The study included 27 patients (54 eyes) with a refraction of -3.00 to -6.50 diopters who received traditional PRK in one eye and LASEK in the contralateral eye. Patients were monitored daily until reepithelialization was complete, applying 1 drop of antibiotic (ofloxacin/ chloramphelicol) QID and an artificial tear (dexatran /hypromellose) every 2 hours until reepithelialization was complete. Reepithelialization was complete on the fourth day for PRK and on the fifth for LASEK. The mean for PRK was 3.18 ± 0.50 days (range: 2 ~ 4 days) and for LASEK 3.64 ± 0.63 days (range: 3 ~ 5 days), without being statistically different (p=0.100).

For the present protocol, subjects treated with multidose Lagricel® PF are expected to show complete reepithelialization by day 3 of treatment.

The sample size was calculated using the equivalence equation between two means [67]. To prove that Lagricel® PF multidose is equivalent to its comparator, considering a power of 80% (β), a significance level of 0.05 (α), and a test margin (δ) of 0.5, based on the following working hypotheses:

$$H_0: |\mu_A - \mu_B| \geq \delta$$

$$H_1: |\mu_A - \mu_B| < \delta$$

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

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

The calculation to estimate the sample size and power was performed using an online tool and following the equations [68]:

$$n_A = kn_B \; y \; n_B = \left(1 + \frac{1}{k}\right)\left(\sigma \frac{z_{1-\alpha} + z_{1-\beta}}{|\mu_A - \mu_B - \delta|}\right)^2$$

$$1 - \beta = 2[\theta(z - z_{1-\alpha}) + \theta(-z - z_{1-\alpha})] - 1, z = \frac{|\mu_A - \mu_B| - \delta}{\sigma\sqrt{\frac{1}{n_A} + \frac{1}{n_B}}}$$

Where:

k= n_A/n_B is the relationship between groups

σ is the standard deviation,

Φ is the function of the standard normal distribution

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

α is the Type I error

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

δ is the test margin

According to the previous calculation, the result was 42 eyes (cases); this calculation was increased by 15% to account for possible losses (6 cases). The total suggested sample size is 96 subjects (48 cases per arm), who will provide their OD 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 Judgment by the International Tribunal of Nuremberg, 1947. Belmont Report, National Commission for the Protection of Subjects of Biomedical Research and Conduct, 1979. It will be conducted in accordance with the scientific and technical requirements necessary for the registration of medicines for human use of the International Council on Harmonization (ICH). International Ethical Guidelines for Biomedical Research Involving Human Subjects of the Council for International Organizations of Medical Sciences (CIOMS , 2002). International Ethical Guidelines for epidemiological studies of the Council for International Organizations of Medical Sciences (CIOMS , 2008). The Research Ethics Committee and the Research Committee will evaluate the protocol before conducting the study and will issue their approval or any possible modifications for its implementation. These Committees must be notified of any significant changes to the protocol. In addition to the above, the current regulations of the regulatory authority must also be complied with.

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

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

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

The study is considered to be research with greater than minimum risk, in accordance with the Regulations of the General Health Law on Health Research, Title Two, Chapter I, Article 17, Section III, published in the Official Gazette on January 6, 1987 / Last reform published DOF 04/02/2014.

11.2 Amendments to the protocol

The amendment process will be relevant when there is a need to make any changes to a document that is part of the research project or protocol, due to changes in the methodological structure, replacement of the principal investigator, or the identification of risks to the research subjects. Documents that may be amended include: the protocol, informed consent letter, researcher's manual, patient documents, measurement scales, and activity schedule.

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

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

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

11.3 Early termination of study

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

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

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

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

11.4 Informed consent

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

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

11.4.1 Obtaining

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

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

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

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

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

If the research subject does not know how to sign, he will print his fingerprint and another person he designates will sign his name.

The IP must also sign and date this consent.

The ICF must be signed in duplicate by all involved parties 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 designated staff member must document the process of obtaining Informed Consent through a detailed medical note, specifying the signed version, the date the document was signed, and how the process was carried out.

11.5 Special considerations

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

11.6 Modifications to informed consent

Any change to the ICF constitutes 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 written approval from the Research Ethics Committee and the Regulatory Body (as applicable), except for an amendment that is required to eliminate an immediate danger to the study subjects.

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

11.7 Confidentiality

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

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

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

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

The researcher will complete and maintain a subject selection record, as well as the identification and enrollment list of each subject participating in the study. The researcher agrees to grant on-site access to the auditor and/or representatives of the Competent Authorities. The information will be processed in compliance with the Federal Law on the Protection of Personal Data Held by Private Parties.

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

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

11.8 Conflict of interest

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

11.9 Declaration of interests

The PI agrees to declare his or her financial interests and conflicts of interest prior to the start of the study.

11.10 Access to information

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

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

11.11 Ancillary and post-study care

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

12. Biosecurity aspects

12.1 No Biosecurity Implications

This protocol, with title: " Phase IV clinical study to evaluate the efficacy of Lagricel® Ofteno PF compared to Thealoz® Duo in corneal reepithelialization following PRK (Photorefractive Keratectomy)", and number: SOPH037-0120/IVDOES NOT HAVE BIOSECURITY IMPLICATIONS, since infectious-contagious biological material will NOT be used; pathogenic strains of bacteria or parasites; viruses of any type; radioactive material of any type; genetically modified animals and/or cells and/or plants; toxic, hazardous or explosive substances; any other material that puts the health or physical integrity of the research center staff or research subjects at risk or affects the environment. Likewise, it is declared that no cell, tissue or organ transplant procedures, or cell therapy will be carried out in this project, nor will laboratory, farm or wildlife animals be used.

13. Publication Policy

13.1 Final report

Once the statistical analysis is complete, the final report will be prepared with the results obtained by the Medical Management of Laboratorios Sophia, SA de CV. This report will be prepared following the recommendations of the ICH E3 Guide . Communication of results

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

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

13.2 Publication of the results

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

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

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

Authorship assignments for publications are the sponsor's responsibility. However, express authorization from those invited to participate as authors is required. Authors have the right to review the manuscript prior to publication, as well as to provide comments and suggestions. Such comments must be submitted within the first 15 calendar days of receipt of the project.

14. Financing and insurance

14.1 Compensation to study participants

Subjects participating in the study will not receive financial compensation for their participation. However, subjects will receive travel expenses for each scheduled visit they attend on time. This amount, as well as the amount, will be specified in the informed consent form.

14.2 Study insurance

In accordance with current local regulations, Laboratorios Sophia SA de CV has contracted a civil liability insurance policy to fulfill its responsibility to provide the medical treatment and compensation to which a subject would be legally entitled in the event of damages directly caused by this research.

In the event of a medical emergency, the research center must have the personnel, materials, equipment, and procedures for its immediate management.

15. References

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