*Excelencia en oftálmicos*

# Efficacy and Safety of the Ophthalmic Solution PRO-087 versus Systane ® Ultra and Systane ® Ultra Preservative Free on Tear Film Dysfunction Syndrome from Mild to Moderate

## Clinical trial

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| **Drug Product ID** | PRO-087 |
| **Drug Product of study:** | 0.1% sodium hyaluronate, preservative-free 0.18% chondroitin sulphate |
| **Therapeutic indication:** | Lubricant for ocular surface |
| **Development phase:** | Phase IV |
| **Protocol ID:** | S0PH087-0415/IV |
| **Sponsor:** | Laboratorios Sophia, S.A. de C.V. |
| **Version:** | 2 |
| **Date:** | february 2016 |

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# 3. Background Information

The tear film is a system with protective, nourishing and optical functions on the eye surface, this is due to its parts that, even though they are produced on different ocular structures, bind and perform synergistically the daily requirements of it. Its quality and production will be directly affected when any of the involved structures is damaged or in poor condition.

Its main components are outlined on an emulsion of 3 layers: a lipid, an aqueous and a mucinous, which together provide enough properties to keep the viscosity, consistency, adherence and osmolarity enough to flow over the ocular surface uniformly; when it is interrupted the integrity of such cape, it is called tear film breakup time (TBUT).[8,9]

After the alteration of any part of the tear film an inflammatory process begins, which will keep chronically altering the production glands system, and will create a continuous cycle between inflammation and dysfunction of the tear film, which will increase according to the evolution time.[1]

The Dry Eye Workshop Study Group (DEWS) considers the dry eye or Tear Dysfunction Syndrome (TDS) as a multifactorial impairment of the tear film and the ocular surface, which happens with ocular damages, visual fluctuations, tear film instability, and potential damage on the ocular surface; the main pathogenic event of the TDS comes from hyperosmolarity and tear film instability.[1,8] Hyperosmolarity causes a series of injuries on the epithelium of the ocular surface, by activating many inflammatory events and releasing various proinflammatory cytokines.[2]

This epithelial damage includes the death of epithelial cells by apoptosis phenomena, loss of goblet cells and alteration of the expression of the surface mucins, which makes a greater instability on the tear film, a phenomenon that may be started by other etiologies, apart from the hyperosmolarity; i.e. xeroftalmia, ocular allergy, exposure to preservatives or the use of contact lenses. The TDS includes two key physio pathological factors: Tear film hyperosmolarity and ocular surface inflammation. The lacrimal functional unit (LFU) is responsible of maintaining the integrity of the tear film. The LFU is made by: Main and accessory lacrimal glands, the ocular surface, eye lids and nerves (sensory, motor and autonomic) which interconnect them (reflex arc nervous system). The afferent route is measured by the trigeminal nerve, while the efferent impulses are conducted by the facial nerve. The facial parasympatethic endings regulate the tear composition and keep a proper balance of the aqueous, mucinous and lipid components. Motor endings, on the other hand, are responsible for the hydrodynamic aspects, which will allow a proper lightening, distribution and evaporation of the tear film.

The main causes of the tear hyperosmolarity are the diminishing of the tear aqueous component, associated to the tear gland impairment and/or the increasing on the tear evaporation, favored, overall, by adverse environmental conditions (low environmental humidity, air conditioning, smoke, contamination, autoimmunity pathologies affecting the gland integrity, chronical use of contact lenses, etc.).[3] Increasing of tear evaporation also increases on cases of Meibomian gland dysfunction, which produce an alteration of the lipid component of the tear film. Reduction of the tear aqueous flow has been related with age,[4,5] reduction of the androgen levels and the effect of some systemic drugs like anticholinergics, anxiolytics, antidepressant, antihistamines, antihypertensive, diuretics, which have been associated with dry eye, as well as with the inflammatory damage over the tear gland that occurs on the Sjogren Syndrome, which destroys the gland tissue.[6]

Changes on the corneal sensibility and a reduction on the tear discharge have been reported on patients who underwent a LASIK surgery.[7]

New evidence outstands the consequences of the use of drugs with preservative when used over large time periods; such consequences are not only new facilities signs and symptoms, but also the worsening of those already present.

The need of managing sterile treatments that aim at the no contamination of the drugs drives the use of preservatives like benzalkonium chloride (BAK), which has many advantages that meant the solution of past problems with ophthalmic formulations, i.e. contamination and later proliferation of diseases, loss of action of the active substances, storing, etc. Among the advantages of BAK are its bacterial action – since it is a cationic surfactant- and its action in alkaline media – optimal at 37 °C; these characteristics make it a candidate as the most effective and widespread preservative, it reacts less against Gram positive and negative; nevertheless, it can increase when combined with 0.1% EDTA.[8,9]

As a quaternary ammonium, it has a detergent action, lysing the bacterial walls, and it is also a great fungicide. Its concentrations more commonly used by the pharmaceutical industry vary from 0.004% to 0.025%; with these it is possible to accomplish the requirements suggested on the International Pharmacopoeia, which request the preservative to be able to eliminate bacterial colonies within 14 days, and fungi colonies over a time period from 3 to 4 weeks.[8]

It has been acknowledged with properties that increase the corneal epithelium permeability, improving the penetration and action of the drug active substances; however, different studies have demonstrated that the availability of the drugs on the anterior chamber and the surrounding tissues has no relation with the presence of BAK nor has a greater efficacy.

Despite the advantages previously shown, it has disadvantages with the chronic use, such as a toxicity by a cumulative effect, since it has been reported that after one instillation of 30 µl of 0.01% BAK this can still be present even 180 hours after its application on the ocular surface of rabbits. It has a shelf life on the outer tissues of 20 hours, and of 11 hours on the deeper tissues. This increases with repeated instillations, since it depends on the washout through the tear film.[8,9]

Prevalence of the TDS, or Dry Eye Disease, is estimated from 15 to 34% on people over 65 years; this is an important data for it can mean bias on studies related with this ailment and its etiology, but mostly, it represents a group of people whose symptoms, related with the TDS, will remain with the long-term use of lubricants with preservatives.[1,8]

Signs and symptoms of TDS, mainly conjunctival hyperemia, tearing, photophobia, burning and sense of a foreign body, are greater on patients using ophthalmic drugs with preservatives. This has been shown on multicenter studies, with significant results, where a prevalence of the signs and symptoms of a 30 to 50% was found.[8]

It is possible to evaluate the clinical impact of these symptoms by different methods. The Schirmer Test reported a reduction of the tear production on 61% of the patients. The TBUT decreased on 78% of patients, fluorescein or lissamine green ocular surface staining show deficiencies on up to 22% of subjects.

Similarly, the impact on the daily life is significant. On a report, where the adherence to the treatment of patients using 1 or more ophthalmic drops with preservative was evaluated, it was found that 65% of the subjects has adverse events related to BAK, with hyperemia and sense of a foreign body as the first symptoms on 48% of the subjects.[1,25]

Everything was related with the fact that at least 48% of the patients suspend the treatment due to ocular discomfort.[25]

The long-term importance of toxicity caused by preservatives is evidenced with conjunctive and underlying tissues ultrastructural changes, i.e. increase of the amount of fibroblast on the own subepithelial substance, fibrosis and pseudopenfigoide reactions. Such changes have been related with the post-surgical tissue behavior in different procedures of patients with chronic diseases who used ophthalmic solutions with BAK for long periods, where satisfactory recovery, in terms of inflammation and wound healing, is not always as expected.

The most important short-term cell and structural changes are depletion of population of goblet cells, which are reduced on the ocular surface 50% after just 1 month of instillation of ophthalmic solutions with preservative. Likewise, instillation of 1 week on healthy patients has already a reduction on TBUT. The 6 months follow-up showed that 60% of subjects had a reduced TBUT, besides an alteration on the lipid layer.[8,9]

The pro-inflammatory cell migration of the innate response promotes secondary immunological reactions. The main molecules found on the conjunctival substance and Tenon’s capsule are antigens HLA-DR and pro-kinetic molecules ICAM3. As a result, there exists a significant increase of CD-20/22/23, as well as CD-4 (Th2).

There are other immuno-histochemical markings that have been used to demonstrate the histological changes and to evidence the weakening of epithelial bonds with cell layers, before activating the apoptosis process. On the left column of figure 1 we can appreciate the tissues unexposed to BAK, and on the right, after having been exposed to BAK for 6 months:

Markings appointed (Figure 1) are ocludine (bonding proteins), present on epithelial cells, ICAM1 (adhesion and conduction), expressed when TUNEL (late apoptosis), caspase (early apoptosis), Ki67 (fibrillary proliferation).

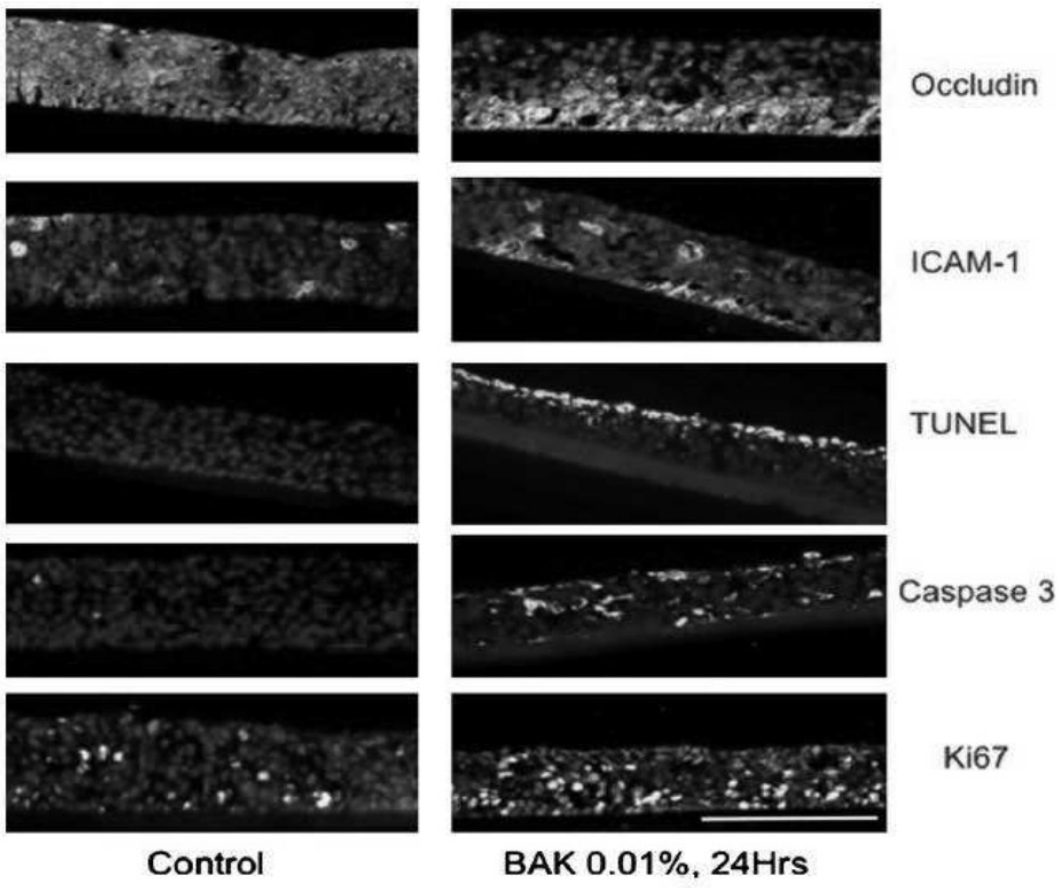



Figure 1. Immuno-histochemical markings on conjunctival tissue.[8]

Bensoussan et al., on a group of 45 patients with 1 year using antigalucoma with preservatives and lubricants also with preservative, performed a flow cytometry on conjunctive samples of subjects treated vs samples of healthy subjects. Besides of confirming the increasing of HLA-DR and CD4/22/23 the presence of IL-8, IL6 and acute inflammatory response cytokines was highlighted (Figure 2).

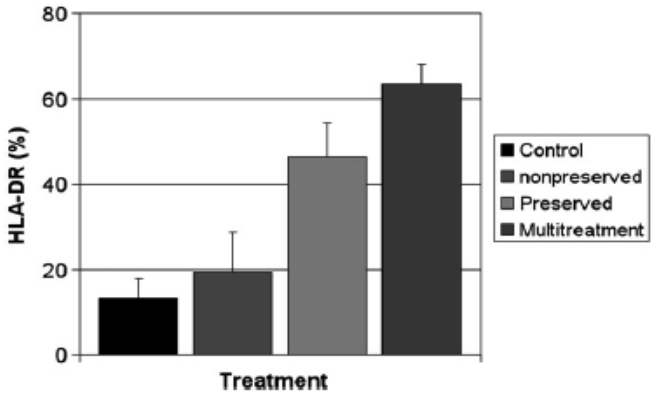



Figure 2. Immunological markings obtained by flow cytometry on subjects treated with preservative vs. healthy.

Long-term changes include entities as[9]:

- Corneal desepithelization
- Loss of epithelial microvilli
- Subconjunctival fibrosis
- Reduction on the number of mucous cells (goblet cells)
- Epithelial keratinization
- Squamous metaplasia
- Increase of desmosomes
- Epithelial Bullosa dystrophy
- Subepithelial fibrosis
- Lymphocytes and subepithelial plasma cells increase
- Basal membrane thickening

Each has a course and a different onset during the evolution of the ailments; it is important to highlight that they have multifactorial etiologies, but the common booster: the chronic use and cumulative effect of preservatives on the ophthalmic solutions used.

### *3.1 Definition, Diagnosis and Seriousness of the Tear Film Dysfunction*

On 2007, during the “Dry Eye Work Shop” (DEWS) the Dry Eye Disease was defined as a multifactorial disease of the tear film and ocular surface, resulting in discomfort symptoms, visual acuity reduction and tear film instability, together with hyperosmolarity of it and inflammation of the ocular surface.

The clinical diagnose starts when identifying the characteristic signs and symptoms of the ocular surface inflammation, corneal and conjunctival staining, epithelial defects, etc. Nowadays, in order to identify not only the advanced cases where signs are already listed, but also mild to moderate cases, different markings have been evaluated and standardized, which, depending on its presence and intensity, determine the seriousness of the syndrome and, therefore, its treatment. On 2013, ODISSEY group evaluated 14 markings and determined its influence on the clinical dosage form and its correlation with the seriousness of the patient symptoms. The evaluated marking were the following:[27]

- Fluorescein corneal staining
- Tear drop hyperosmolarity
- Schirmer Test
- Impression cytology
- Filamentous keratitis
- Conjunctival staining
- Visual acuity decreasing
- Palpebral and/or Meibomian inflammation
- Blepharospasm
- TBUT
- Aberrometry

- Corneal confocal microscopy
- Inflammatory biomarkers (MMP9, cytokines, HLA-DR)
- Clinical charts refractory to previous treatments

On many occasions, even though all can be present on every subject with tear film dysfunction, previous markers or criteria do not agree with the actual ocular damage, or, in mild to moderate cases, they are not totally perceptible by the patient.

Tests as the ocular surface disease index (OSDI), or other tools, have demonstrated an acceptable correlation with the syndrome clinical seriousness, as well as with the corneal staining ($r^2$ = 0.41 vs 0.43, respectively), so even if it is useful to distinguish true cases of TFD from occasional symptoms, that does not give a true correlation of the seriousness of the case.[22]

Previous data lead to the performance of this study, in order to assess the correlation of all markers individually or combined, and to be able to determine which were more useful to specifically determine and distinguish the seriousness or grade of the ocular surface damage.

Alvez M. et al. evidences that the best proof combination to achieve the greatest sensitivity, conjugating its results was to perform an OSDI, a fluorescein TBUT and a Schirmer test, which gives a combined sensitivity of 100% (95% IC 97.5 – 100), a specificity of 95% (95 IC 75.1 – 99.9%) and precision of 99.3% (95% IC 96 – 99.9). Previous findings enforce the importance of the most commonly tests used by costs, accessibility and reproducibility.[26]

This concludes that though tear film dysfunction diagnosis algorithms are not stablished, basic elements to perform it are as follows:

- TBUT < 10 seconds
- Schirmer test less than 100 mm (5 minutes)
- OSDI score > 20 and < 30
- Fluorescein or rose Bengal corneal staining score ≥ I (Oxford scale)
- Osmolarity > 310 mOsm

Any previous marker, added to each subject clinical chart, established the tear film dysfunction syndrome diagnosis.

Charts with OSDI > 30, a score equal or > to III on an Oxford scale with fluorescein staining and altered tear film break-up time are considered as serious.[27]

### *3.2 Treatment for the Tear Film Dysfunction Syndrome*

Directly related with seriousness. Surgical handling consists of temporary (collagen) or extended (silicone) tampons to hold tear secretions. The permanent closure of tear duct can be made by electrocautery or laser. Pharmacological handling contemplates the use of artificial tears as first line therapy, since the liquid replacement is pretended, notwithstanding, mucine restauration takes a bigger challenge. Recently, high molecular weight polymers have been used with the purpose of improving and extending the moisturizing surface, as well as imitating the function of the mucine, like sodium hyaluronate (SH) and chondroitin sulphate (CS).

Nevertheless, adverse events related with the use of a preservative agent on the formulation of drugs used to treat the tear film dysfunction syndrome have been reported, like BAK related with the epithelial cells conjunctival and corneal toxicity, due to its emulsifying effect on cell membranes, which produces a tear film and a stimulation resulting of inmuno-allergic reactions.

Due to this, the use of formulations without preservative agents has been privileged, especially on patients requiring sequential applications and that have a greater sensitivity.
Laboratorios Sophia formulation Pro-087 is made of SH and CS, it does not have BAK, and its additives have been especially combined to optimize the effect of glucosaminoglucane and to decrease the risk of adverse events and toxicity.

### *3.2.1 Sodium Hyaluronate (SH):*

It is a hyaluronic acid sodic salt. It is a glucosaminoglucane, disaccharide, biopolymer, formed of an alternating sequence of N-acetyl-D-glucosamine and glucoronate on lineal chains. On physiological solvents forms spirals, a configuration determined by its viscosity. It is a constituent of the extracellular matrix, conjunctive tissue, vitreous humor, umbilical cord, synovial fluid, skin, etc. it can be formed by more than 10,000 pairs of disaccharides. At concentrations of over 0.1%, sodium hyaluronate forms a network. Diffusion rate trough the network is reversely related to the polysaccharide molecules size, which are stable. Proteoglycans give mechanical and elastic properties. Many authors have reported that SH has a high capacity to hold water; it has been established that 1 gr of SH can hold up to 6 L of water.[10]

Sodium hyaluronate is synthetized on the inner face of the plasmatic membrane as a lineal polymer, in contrast to other glucosaminoglucanes, which are synthetized by Golgi enzymes.

Enzymes for the synthesis of sodium hyaluronate are hyaluronate synthetasas, which are part of the plasmatic membrane; and glucosiltransferases, which coordinately polymerize and translocate the sodium hyaluronate out of the cell towards the extracelular matrix.[11]

### *3.3 PHARMACOLOGICAL PROPERTIES*

#### *3.3.1 Pharmacokinetics on the Eyeball*

**3.3.1.2 Route of administration:** ophthalmic. **Release:** conventional. **Absorption:** it has not been reported an absorption of SH through the cornea when applied over the ocular surface. Pharmacokinetic studies performed on patients with dry eyes showed that sodium hyaluronate solution reached the maximum concentration in 10 minutes and it is widely **distributed** on the ocular surface; it is **biotransformed** by hyaluronidases, it is **eliminated** from here through the lacrimal sac and duct without intraocular absorption, on approximately 45 minutes.[12] Based on the results of preclinical investigations on rabbits developed by Laboratorios Sophia SA de CV, **ocular shelf-life** is correlated with the formula volume.[13]

#### *3.3.2 Pharmacokinetics from Systemic Administration*

**Administered** endovascularly, SH is **absorbed** by the liver endothelium receptors and degraded to monosaccharides and oxidation products. **Distribution**: systemic, wide. **Metabolism**: biotransformed by hyaluronases, mainly on the hepatocyte. **Elimination**: biliary. **Plasmatic shelf-life** is of 2.5 to 5.5 minutes.

#### *3.3.3 Pharmacodynamics*

SH has many functions, as cell adhesion, migration and proliferation. It has been reported that sodium hyaluronate participates on many events during morphogenesis and differentiation.[14]

Recently Quiao H., et al. researched the possible hyalocites physio pathological role on the eye. They proved that hyalocites could participate not only on vitreous diseases proliferation, but also on immunological disorders. It was observed by a micrography study that hyalocites are free cells, fully separated of the inner limiting membrane, surrounded by collagen fibrils on the vitreous cortex; It was first seen on rodents that hyalocites are derived from bone marrow cells and with phenotypic characteristics similar to macrophages, and for its derivation and location contribute to hold the vitreous.[15]
SH improves the ocular surface condition, stabilizes the precorneal tear film and reduces the symptomatology intensity associated with the conjunctival sac and a prolonged contact with the cornea.[16]
Increases the ocular residence of many drugs like: pilocarpine, tropicamide, timolol, gentamicine, tobramicine, which determines a rise of the bioavailability.
There is a hypothesis that the re-epithelizing effect of SH on the cornea is related with the stimulating action of epithelial cell migration, and with the nil toxicity on the cornea stroma, once the epithelium is debrided during surgery.[17,18,19]

### *3.4 Chondroitin Sulphate (CS)*

It is part of the glucosaminoglucanes group. As sodium hyaluronate, it is a mucopolysaccharide, found on the extracellular matrix of connective tissues, including the vitreous, cornea and aqueous humor. Glucosaminoglucanes are made from aggregates of high molecular weight, named proteoglicanes. These contribute to add mechanical and elastic properties to the products that have CS.[20]

Chondroitin sulphate monomer is a disaccharide compound of N-acetilgalactosamine and N-glucoronic acid. Sulphate group is fixed on the galactosamine, on 4 and 6 position, which explains the existence of 2 isomers of chondroitin sulphate.

### *3.4.1 Pharmacokinetics on the eyeball*

**Route of administration**: ophthalmic. **Release**: immediate. Widely distributed on the ocular surface. **Absorption**: from a formulation administered topically it has been determined that there is no absorption through the cornea. **Metabolism**: by reactions of biotransformation of phase I, oxidation and reduction. **Elimination:** through lacrimal system.

### *3.4.2 Pharmacokinetics from oral administration*

In case of oral **administration**: the agent is quickly released and has a high absorption. **Distribution:** widely, predominantly to an articular level. **Elimination:** the systemic clearance of CS is of 30.5 ml/min or 0.43 ml/min/kg. The average shelf-life time ranges from 5 to 15 hours, depending on the experimental protocol. Most of the whole drug and its metabolites are eliminated by the kidneys.

### *3.4.3 Pharmacodynamics*

Applied on the eye, both CS and SH increase tear film stability and reconstitution and contribute to the improvement of ocular microenvironment. Chondroitin sulphate favors the epithelium reestablishing in corneal ulcers, inhibiting the collagenase and stimulating the cell growth. It facilitates the maintenance of a smooth surface that allows a regular light refraction and to lubricate the ocular surface to enable blinking. It also has an important role on the retinal histogenesis and acts as a regulator of neuronal pattern on the retina.

In other systems, CS is one of the main elements of the cartilage, which bonds to a central protein, making the proteoglycan that grants the cartilage its mechanical and elastic properties.

The CS therapeutic use on arthrosis patients is due to an anti-inflammatory activity on the level of cell parts of the inflammation (in vivo), the proteoglycans synthesis stimulation (in vitro) and endogenous hyaluronic acids (in vivo), and to the reduction of chondrocytes catabolic activity (in vivo), inhibiting some proteolytic enzymes as collagenase, elastase, proteoglycanase, phospholipase A2, N-acetilglucosaminidase, etc.

Tears are 98.2% water and 1.8% solids. The high tear water percentage is due to the need of lubricating the conjunctiva and corneal surface. Water evaporation within blinking may have an influence on the tear film concentration. It has been observed that water evaporation speed from precorneal tear film, undamaged through the lipid outer layer, is of $8X10^{-7}$ $cm^{-2}.seg^{-1}$. On a 10 seconds interval (between 2 consecutive blinkings) the tear film thickness reduces approximately 0.1 mm, which determines an increase of water concentration of almost 1 – 2%. Instead, solutes concentration increases approximately by 20%.

PRO-087 Formulation, when combined with SH and CS, increases the residence time of the tear hydric proportion, and forms a homogeneous film, holding moisture and cell viability. It is then possible to identify the formation of the PRO-087 formulation.

*3.5 Qualitative formula PRO-087*

| Table 1. Formation of Pro-087 formulation | | |
|---|---|---|
| **Active substance** | **Amount mg/ml** | **Role** |
| Sodium sulphate chondroitin | 1.80 | Active substance |
| Sodium hyaluronate | 1.00 | Active substance |
| Additives | Amount mg/ml | Role |
| Pharmaceutical form: solution q.s.(1) | Not exhibited | Not exhibited |
| Table 1. Formation of Pro-087 formulation. Substances with demonstrated pharmacological substances are shown, as well as additives of the formulation per milliliter.<br>Abbreviations: q.s. = *quantum satis* (amount which is needed) ml = milliliter | | |

# 4. Rationale

The use spectrum of lubricant drops for ocular surface in the population and with certain pathologies ranges from drugs sold over the counter to the specific compounds to replace the different layers of the tear film; many of them will be used for periods longer than 6 months and in the case of chronic diseases perhaps for years. According to recent meta-analysis, it is estimated that annually about $ 540 million dollars are spent globally; they are the first line treatment for ocular symptoms related with the tear film dysfunction, on healthy subjects, with a prevalence of 25 to 35% on people over 60 years old and on more than 6% of people over 40 years old. If we add the occasional symptoms that relate or depend on some work or occupational situation and people who use it by intermittent periods throughout their life, the population spectrum who will have access to these drugs is very large.

An estimated 50% of patients diagnosed with tear film dysfunction without concomitant diseases will use more than two ophthalmic solutions in 5 years of treatment.

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

# 5. Objectives

## *5.1 MAIN OBJECTIVE:*

**To evaluate the effectiveness of preservative-free ophthalmic formulation PRO-087 (by Laboratorios Sophia, S.A. de C.V.) to restore the anatomical and physiological characteristics of the ocular surface, as well as its distribution and the characteristics of the mild to moderate tear film dysfunction syndrome compared to Systane ® Ultra and Ultra Systane ® preservative free (by Laboratorios Alcon, S.A. de C.V.).**

## *5.2 SPECIFIC OBJECTIVES:*

**5.2.1** To evaluate the safety of the preservative-free ophthalmic formulation PRO-087 (by Laboratorios Sophia, S.A. de C.V.) on the corneal and conjunctival epithelium, intraocular pressure, and structures of anterior and posterior segment in patients with tear film dysfunction syndrome from mild to moderate.

**5.2.2** To determine the correlation between improvement of clinical status and perceived improvement of symptoms of each participant in each study group.

**5.2.3** To compare the qualitative and quantitative histological status of the ocular surface before and after the pharmacological intervention in each study group to determine the evolution under the intervention of PRO-087.

**5.2.4** To evaluate the quantitative rating of tear film production by the Schirmer Test throughout the study.

**5.2.5** To qualitatively assess the tear film production by measuring the tear film break-up time stained with fluorescein and cobalt filter.

# 6. Summary

| | |
|---|---|
| Drug product ID | PRO-087 |
| **Study drug:** | 0.1% sodium hyaluronate, free-preservative 0.18% chondroitin sulphate |
| Therapeutic indication: | Ocular surface lubricant |
| Protocol ID: | **S0PH087-0415/IV** |
| **Sponsor:** | Laboratorios Sophia, S.A. de C.V. |
| **Version:**<br>**Date:** | 2<br>February 2016 |
| **Study title** | Efficacy and Safety of the Ophthalmic Solution PRO-087 versus Systane ® Ultra and Systane ® Ultra Preservative Free on the Tear Film Dysfunction Syndrome from Mild to Moderate<br>Clinical trial |

| | |
|---|---|
| **Main objective** | To evaluate the effectiveness of preservative-free ophthalmic formulation PRO-087 (by Laboratorios Sophia, S.A. de C.V.) to restore the anatomical and physiological characteristics of the ocular surface, as well as its distribution and the characteristics of the mild to moderate tear film dysfunction syndrome compared to Systane ® Ultra and Ultra Systane ® preservative free (by Laboratorios Alcon, S.A. de C.V.). |
| **Study design** | Controlled, randomized, double-blind, masked clinical study, comparing the safety and efficacy of preservative-free PR0-087 vs Systane Ultra with preservative and Systane Ultra preservative free, in subjects with mild to moderate tear film dysfunction syndrome, for a period of 90 days plus 15 days of remote surveillance, in which one of the three agents will be administered (PR0-087, Systane® Ultra or Systane® Ultra preservative free) with a q.i.d. dosage. in both eyes, with regular follow-up visits (5 overall). |
| **Variables to evaluate** | Best-corrected visual acuity<br>Intraocular pressure<br>Ocular surface<br>Anterior segment examination<br>Posterior segment examination<br>Tear film break-up time<br>Schirmer test<br>Corneal depithelization<br>Goblet cells count<br>Adverse events |
| **Groups** | Subjects with a clinical diagnosis of mild to moderate tear film dysfunction syndrome between 18 and 90 years old, without concomitant eye diseases nor requiring different treatments of any of the three interventions in this study They will be randomized in 3 groups where PRO-087, Systane® Ultra o Systane® Ultra preservative free will be administered. |

# 7. STUDY DESIGN

### *7.1 Design*

Controlled, double-blind, randomized, clinical trial comparing the safety and efficacy parameters between a group of subjects with a diagnose of mild to moderate tear film dysfunction syndrome under a regimen of ophthalmic solution lubricant drops PRO-087 versus subject under Systane® Ultra or Systane® Ultra preservative free, with the same diagnosis, with a follow-up of 90 days.

### *7.2 Sample:*

288 patients with tear film dysfunction, classified as mild to moderate, will be included and randomized into 3 groups; the first being treated with PRO-087 ophthalmic solution, the second with Systane® Ultra ophthalmic solution, and the third with ophthalmic solution Systane® Ultra preservative free.

### *7.3 Blinding:*

The double-blind study is a procedure in which the patient and the treating doctor ignore to which intervention group the study patient was assigned. To achieve the blinding of both the drug in research and both comparator drugs, these will be labeled in the same way (masking). Besides the figure of an unblinded pharmacist, who is responsible for the delivery of the medication to the patient, will be added. Blinding codes are protected by an outsider appointed by the study sponsor. The codes are also available in the research center (fully sealed), so that they can be consulted by the investigator in case a subject presents a serious adverse event, prior authorization from the study sponsor; the blinding also continues rigorous during the data analysis and interpretation.

### *7.4 Pharmacological Intervention*

The pharmacological intervention will be the instillation of the ophthalmic solution in the bottom of the conjunctival sac during the waking period, in any of the following study groups:

1. Preservative free PRO-087 ophthalmic solution. Dropper bottle. Multidose

   1 drop every 4 hours for 90 days.
2. Systane® Ultra ophthalmic solution. Dropper bottle. Multidose.

   1 drop every 4 hours for 90 days.
3. Systane ® Ultra preservative free ophthalmic solution. Single-use vials.

   1 drop every 4 hours for 90 days.

### *7.5 Allocation to intervention groups*

The allocation to the study groups will be held in the visit outlined in the study schedule, randomly, through a specialized software that uses random number tables to achieve a uniform allocation in each intervention group.

### *7.6 Ethic considerations*

This protocol conforms to the Good Clinical Practices (GCP) and principles from the 18$^{th}$ Medical Assembly in Helsinki, Finland in 1964 and the amendments made in Tokyo, Japan 1975, Venice, Italy 1983, Hong Kong 1989 and the 48$^{th}$ General Assembly Somerset West, South Africa in 1996, 59$^{th}$ General Assembly, Seoul, Korea, 2008, 64$^{th}$ General Assembly, Fortaleza, Brazil, 2013, where medical research (clinical research) is contemplated.

Also, according to the specifications of the General Law of Health of Mexico, on research for health, section 17, this study is considered on the subsection III with greater risk to the minimum, by the cell sampling and drug administration. Under this legal framework, the full respect for the person, life and safety is maintained.

**Research Ethics Committee**

The principal investigator will submit the study protocol, informed consent, investigator handbook, materials to deliver to the patient, recruiting materials, and the necessary documents, in accordance with local requirements, to a Research Ethics Committee, supported by the National Bioethics Commission, and with current registration in the Federal Commission for Protection against Sanitary Risks (COFEPRIS).

The study will not start in the investigation center without first obtaining the approval of the Research Ethics Committees and Research Committee concerned, having met the local regulatory requirements, having obtained the signature of confidentiality agreements, economic proposal and the contract signature of each of the senior research physicians.

Information for the subject and informed consent form:

The consent form must be obtained before the patient is treated with any procedure specified in the protocol.

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

The principal investigator (or a member of the research medical team of the site properly specified by the principal investigator in the form of delegation of responsibilities) will provide the prospective participant with all the information relating to the characteristics of the study, its potential risks, benefits, objectives and procedures thereof. This information shall be in a language understandable to the subject, it will be explained to the patient that he/she is entitled to discontinue the participation in the study at any stage, without affecting the relationship with the investigator and/or its future assistance. Informed consent will be placed before the potential participant; he/she must have enough time to analyze each and every one of the abovementioned aspects, and, if in doubt, this will be clarified by the person responsible for obtaining the informed consent. Once the participant agrees to participate in the study, he/she must sign and date the written informed consent in the presence of two witnesses, who may or not have relation with the subject of study, who will be involved during the informed consent process and will sign endorsing that the process is carried out prior to any study procedures, which clearly explained the study information and clarified any doubts, if available.

Should a patient be illiterate, the acceptance will be with the fingerprint, and if the patient is unable to grant a proper written informed consent, a patient representative "legally authorized" can provide such consent in name of the subject, in accordance with the laws and regulations.

*The principal investigator must also sign and date this consent.*

Informed consent should be signed in duplicate by all the involved and two witnesses; a copy will be filed in the investigator’s folder and another investigator will grant the other copy to the participant.

The investigator must document in the patient clinical chart the date on which the informed consent was signed.

At the time that informed consent is obtained, a unique subject identification number will be assigned, this will be used throughout the study to identify the participant.

**Amendment to the "informed consent"**

Any changes made to the "informed consent" constitute an amendment to this document and must be submitted for approval before the Research Ethics Committees, and if applicable, before the competent authorities.

In the amendment, a copy of the new version in the official language or languages of the country will be included.

Such amendments could be implemented only after having obtained the written approval of the Research Ethics Committee and having met with the local regulatory requirements, except for an amendment that is required to remove an immediate hazard to the patients in the study.

Each patient affected by the amendment must complete, date and sign two original copies of the new version. The patient will be given a signed original amendment and the principal investigator shall retain the second one.

**Pregnancy test**

Urine pregnancy test will be taken in the same facilities of the research organization, where the study participant will be provided with a home pregnancy test device, which she will use in private, explaining that she must pour urine on absorbent end of it for 10 seconds; after this she should put the already included plastic cover and let the device stand for 3 minutes on a flat surface with result windows turning up, it is important not to move it during this period.

The result can be seen after 3 minutes and before 15 minutes of conduction. A line is observed in the control window to know that it is working properly, then a pink line will appear in the output window, the intensity may vary, but if present, it is an indicator of a possible pregnancy.

**Confidentiality – use of information**

All documents and information provided to the investigator by the sponsor are strictly confidential. The investigator expressly agreed that the data on their professional and clinical experience, provided to the sponsor on paper and stored in the computer, are for use related with the activities with the sponsor of clinical trials, according to Good Clinical Practice. The principal investigator agrees that he/she and the members of the team will use the information only within the framework of this study, to carry out the protocol. This agreement is mandatory as the confidential information has not been publicly disclosed by the sponsor. The clinical trial protocol provided to the investigator may be used by himself and his colleagues to obtain the patient informed consent for the study. The clinical study protocol, as well as any information taken from it, must not be disclosed to other parties without the written consent of the sponsor.

The investigator will not reveal any information without the prior written consent of Laboratorios Sophia S.A. de C.V., except to the representatives of the competent authorities, and only by their request. In the latter case, the investigator is forced to inform Laboratorios Sophia S.A. de C.V. before disclosing the information to these authorities. The investigator will fill and maintain a logbook of the subject selection and the identification and enrollment list of each patient.

The investigator agrees to give access on-site to the auditor and/or to the representatives of the Competent Authorities. The information will be treated in compliance with professional secrecy.

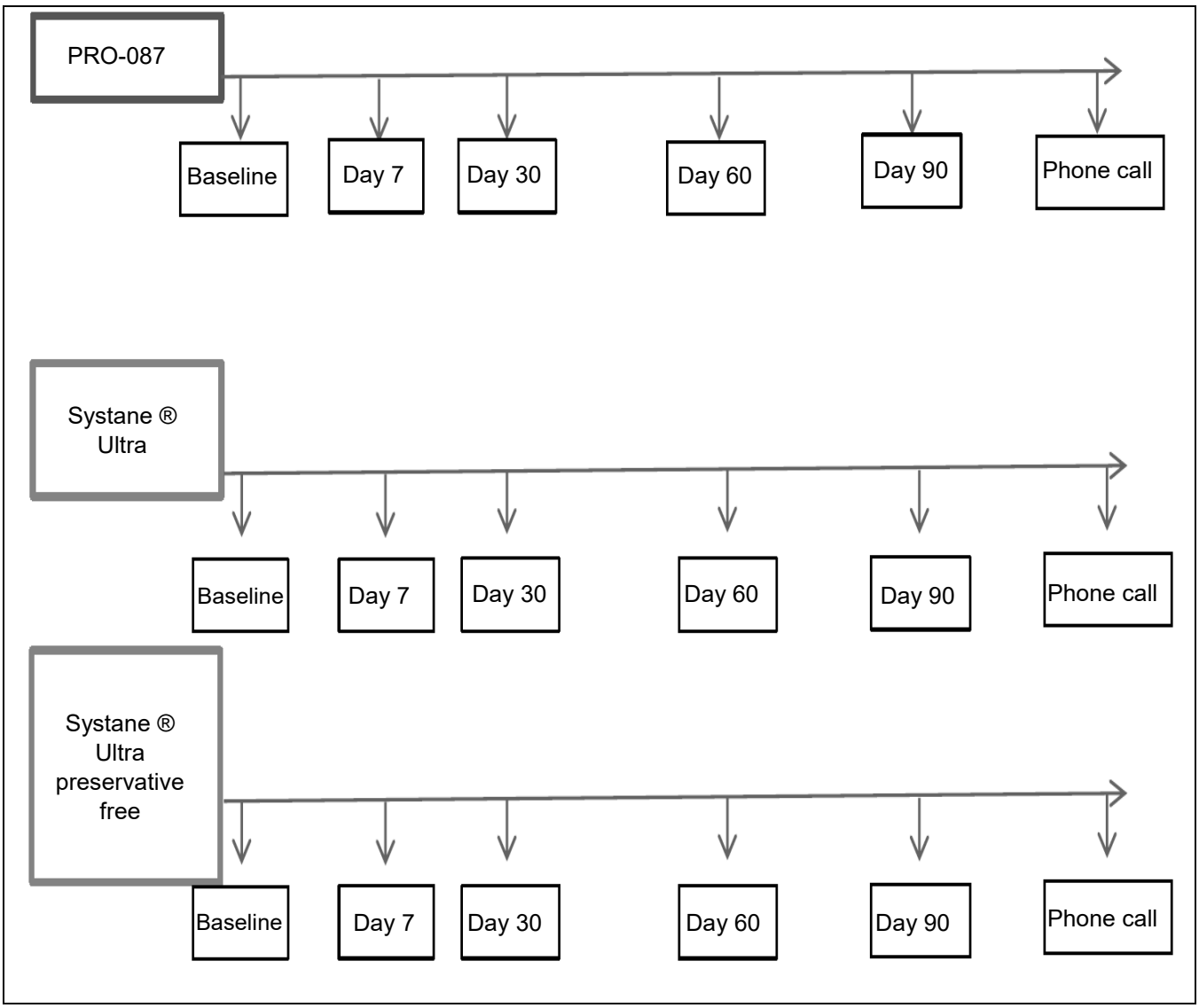
The logbook of subject selection will begin to be filled from the time the investigator determines that a patient could participate in the study (by assessment of the patient's medical history during a visit or a medical record review).

**Center Organization**

Any person to whom the principal investigator delegates, under its responsibility, part of the follow-up study (co-investigator, sub-investigator, nurse) and any other person involved in the study of this center (cardiologist, pharmacist, etc.) will be included in the format of Delegation of Responsibilities.

This document must be delivered at the start of the study and updated in case one of the people involved in the study at the center is changed.

*7.7 Study Plan*



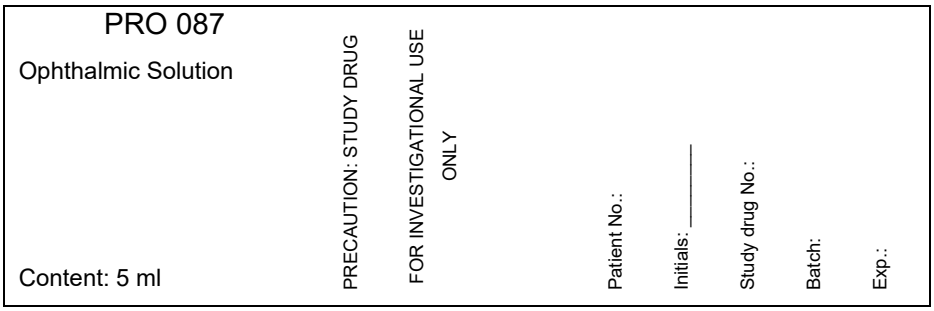
# 8. Drug Product of Study

## *8.1 Codes, labelling and storing*

The dropper bottles will be delivered on its primary and secondary packaging to the different participating research centers; they will come identified with the ID protocol, below the specification of solution or suspension and content in milliliters, among other data, as illustrated below.

### *8.1.1 Primary Packaging*

Sample:



### *8.1.2 Secondary Packaging*

| | | |
|---|---|---|
| Drug product No.:<br>Batch:<br>Expiration date: | PRO-087<br>Protocol: SOPH087-0415/IV<br>Subject No.: _____<br>Subject initials: _____<br>Investigator: _____<br><br>Ophthalmic. Apply according to the protocol indication.<br><br>Keep at room temperature at no more than 30 °C<br><br>Clinical investigation sample<br><br>Investigational drug product | REMOVE LABEL<br>PRO-087<br>Protocol: SOPH087-0415/IV<br>Drug product No. _____<br><br>PRO-087<br>Protocol: SOPH087-0415/IV<br>Drug product No. _____ |

### *8.2 Drug Product of Study*

The formulation PRO-087 is made of the following active agents: 1.80 mg/ml of chondroitin sulphate and 1.00 mg/ml of sodium hyaluronate, as well as the inactive agents: boric acid, decahydrate sodium borate, polysorbate 80, sorbitol, sodium chloride, dihydrate disodic edate, potassium chloride, hexahydrate magnesium chloride, purified water.

It is dispensed in a dropper bottle (which has a container closure system that allows to preserve the solution) for a multidose administration, as a preservative free ophthalmic solution of 10ml.

**Systane® Ultra** is made of the following active agents: 0.4% of polyethylene glycol 400 and 0.3% of propylene glycol, and the inactive agents: aminomethyl propanol, boric acid, hydroxypropyl guar, 0.001% of POLYQUAD® (polyquaternium-1) with conservative, potassium chloride, purified water, sodium chloride, sorbitol, hydrochloric acid/sodium hydroxide to adjust pH. It is dispensed in a dropper bottle for multidose administration, as ophthalmic solution of 10 ml.

**Systane® Ultra preservative free** is made of the following active agents: 0.4% of polyethylene glycol 400 and 0.3% of propylene glycol, and the inactive agents: aminomethyl propanol, boric acid, hydroxypropyl guar, potassium chloride, purified water, sodium chloride, sorbitol, hydrochloric acid/sodium hydroxide to adjust pH. It is dispensed as ophthalmic solution in single-use vials of 0.4 ml.
Pharmacological intervention dosage with drug products of study: 1 drop 4 times daily for 90 days.

### *8.3 Handling of the Drug Product of Study*

The drugs will be shipped from the storage center (located in Laboratorios Sophia S.A. de C.V.) previously weighed and packaged.

**The handling of the drug product of study will be the responsibility of the principal investigator in charge of the research center**, including:

- Receipt and storage of drug product of study. The receipt of the drug product of study must be acknowledged by signing and sending back the corresponding format. The drug product of study should be stored in a secure area with restricted access. Some special storage conditions are requested, such as to keep at room temperature of no more than 30° Celsius, for which the doctor will be provided, on loan during the study, with a thermos-hygrometer that records humidity and temperature. Principal investigator or pharmacist assigned must conduct a thermo-hygrometer reading at least once a day, keeping a record of:
    - Current temperature
    - Minimum temperature
    - Maximum temperature
    - Relative humidity
- Data review registered in the of thermo-hygrometer diary will be performed on the clinical monitor during monitoring visits and, in turn, the previously assigned responsible will perform a reading a day directly from the recorder screen, which will have to write down on the corresponding format.
- Expiration date will appear on each box and label.
- The investigator and/or pharmacist of the research center shall use the treatment delivered only for participating subjects of study.
- Drug product of study count:

The investigator and/or pharmacist of the Research Center and/or a person designated by the study team must fill in real time all the documents that the sponsor provides for the management of the drug product of study.

The study monitor will periodically check the handling and counting of the drug product of study.

At the end of the study, the principal investigator and the study monitor will complete a final inventory of drugs, and will record in the appropriate form.

The study monitor will collect the remaining drug product of study for storage and subsequent destruction, after quantifying the remaining amount in each container.

They must report all faults or damages of the drug product of study or of its packaging to monitor the study. The principal investigator shall notify the monitor all complaints received from a subject.

In case of early return of the drug product of study to the sponsor (removal of the batch), the sponsor will prepare an informative letter to the principal investigator and/or pharmaceutical of the research center. This letter will be sent by people locally responsible of the study to each of the study centers. Upon receiving the letter, the investigator and/or pharmacist will identify the subjects who have a treatment at the time the incident is known, and will contact them immediately. The monitor will organize the return of the drug product of study for the purposes of destruction.

# 9. Study Schedule

| | FOLLOW UP | | | | | |
|---|---|---|---|---|---|---|
| Procedure | Baseline visit | Visit 1 | Visit 2 | Visit 3 | Final visit | Follow-up call |
| | Day 1 | Day 7 | Day 30 | Day 60 | Day 90 | Day 105 |
| Eligibility criteria (inclusion and exclusion) | X | | | | | |
| Pregnancy test (if applicable) | X | | | | X | |
| Informed consent sign | X | | | | | |
| Medical and ophthalmic history (A,B,C). | X | X | X | X | X | |
| OSDI questionnaire | X | X | X | X | X | |
| Code assignment to the subject | X | | | | | |
| Visual acuity and Best-corrected visual acuity | X | X | X | X | X | |
| Conjunctival impression cytology | X | | | | X | |
| Fluorescein staining | X | X | X | X | X | |
| Tear break-up time | X | X | X | X | X | |
| Rose Bengal staining | X | X | X | X | X | |
| Schirmer Test | X | X | X | X | X | |
| Anterior biomicroscopy (D, E, F, G, L) | X | X | X | X | X | |
| Posterior ophthalmoscopy under mydriasis (H, I, J, K) | X | | | | X | |
| IOP measurement | X | | | | X | |
| Treatment delivery | X | | | | | |
| Subject journal review | X | X | X | X | X | |
| Adverse events evaluation | | X | X | X | X | |
| Drug product application | X | X | X | X | X | |
| Drug product devolution | | | | | X | |
| Concomitant drug product evaluation | X | X | X | X | X | |
| Safety phone call | | | | | | X |

### *9.1 Delimitations of the Activities Schedule*

A. Burning
B. Foreign body sensation
C. Tearing
D. Conjunctival hyperemia
E. Chemosis
F. Lens
G. Iris
H. Retina
I. Macula
J. Vitreous
K. Excavation of the optic nerve in decimals
L. Tear film break-up time

# 10. Procedure Description

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### *10.1 Medical History*

It refers to a holistic assessment of all factors affecting the subject's health status. Including information about social, cultural, familial, and economic aspects of the subject's life as well as any other component of the life style that affects health and well-being, emphasizing on medical and surgical background, specifying the start, evolution and, according to the case, ending of the previous ailments.

List the medications taken and dates of last dose; specify the route of administration thereof.

A general physical examination, including vital signs (blood pressure, body temperature, breathing per minute and heart rate) and anthropometric parameters (weight, height and complexion) will be performed.

### *10.2 Ophthalmic Clinical History*

Questions about previous ocular chronic or acute ailments will be asked; specifying time of onset or diagnosis and its evolution.

Optical correction devices used by the patient shall be listed if so, such as frame glasses, contact lenses, magnifying, etc. Similarly, evolution time and use period will be expressed.

Subsequently, topical medications used in the eyes and annexes will be investigated, in case there is any systemic medication, it will be written again in this section if it is directly related to an ocular disease or its annexes (eyelids, orbit, lacrimal route, annexed glands, etc.).

### *10.3 Ophthalmic Examination*

#### *10.3.1 Visual Acuity Measuring*

Visual acuity will be evaluated basally, without optical correction, with a Snellen chart in a location with adequate natural or artificial lighting, and the result will be recorded on the file in fraction according to the number of lines read by the patient. This data will be reported as visual acuity.

Then the visual acuity of the individual with his best optical refraction will be measured and recorded in the same way it was made on the part of the Snellen chart. This data will be reported as best-corrected visual acuity.

Both data will be reported for statistical purposes in logarithmic figures (LogMAR). A conversion table to LogMAR fraction is attached.

#### *10.3.2 Visual Examination Standardization*

- At a distance of 3 m in a proper lighted room the research subject will be evaluated as follows
- This test will be done in the research center :.
- The subject will be asked to sit always in the same place; visual acuity (VA) will be assessed with the scale of LogMAR, if the chart is missing, conversion should be performed (Fig. 10.3.1.1).
- The research subject will keep both eyes open.
- The research subject should gently cover one eye with the occluder provided by Laboratorios Sophia S.A. de C.V., (the same occluder must always be used to cover the eye with all research subjects), while reading aloud the smallest line of letters that he or she can see. This test is done on each eye, one at a time, starting with the right eye (OD).
- The physician should point the line asked to read the research subject.
- The same steps apply for taking best-corrected visual acuity.

| LogMAR | VAR | Snellen (m) | Decimal | Snellen (ft) |
|---|---|---|---|---|
| 1.0 | 50 | 6/60 | 0.10 | 20/200 |
| 0.9 | 55 | - | - | 20/150 |
| 0.8 | 60 | 6/36 | 0.15 | 20/120 |
| 0.7 | 65 | - | 0.20 | 20/100 |
| 0.6 | 70 | 6/24 | - | 20/80 |
| 0.5 | 75 | 6/18 | 0.30 | 20/60 |
| 0.4 | 80 | - | 0.40 | 20/50 |
| 0.3 | 85 | 6/12 | 0.50 | 20/40 |
| 0.2 | 90 | 6/9 | - | 20/30 |
| 0.1 | 95 | - | 0.75 | 20/25 |
| 0.0 | 100 | 6/6 | 1.00 | 20/20 |
| -0.1 | 105 | 6/5 | - | 20/15 |
| -0.2 | 110 | 6/4 | 1.50 | - |
| -0.3 | 115 | 6/3 | 2.00 | 20/10 |

Fig. No. 10.3.1.1 Table of conversion to LogMAR

*10.3.3 Biomicroscopy*

A sequential order of the eyelids and annexes will be taken to the ocular surface and the anterior chamber. The following points should be gathered:

- ❖ Eyelids, eyelashes and gland annexes description

- Description of palpebral conjunctiva, bulbar and fornix: at this point we will grade qualitative characteristics as follows.
- Bulbar hyperemia: It will be related to the images shown in Figure 10.3.3.1 in which different degrees of venous turgor range from very mild, mild, moderate and severe.

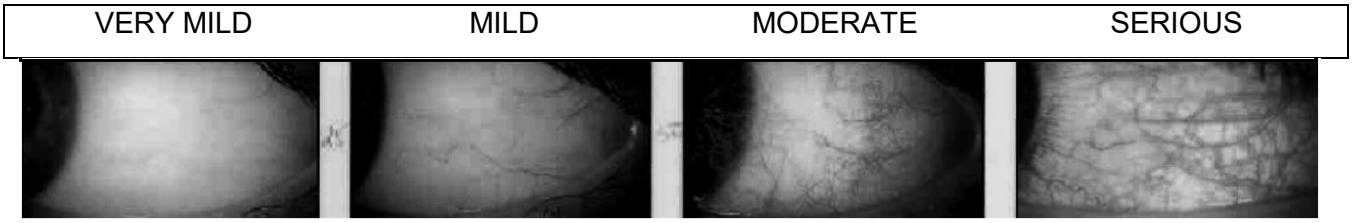



Fig. 10.3.3.1[28]

- Tarsal hyperemia: On the same way, the tarsal hyperemia will be related with the images shown in Figure 10.3.3.2, where the venous turgor in the palpebral mucosa is also shown.

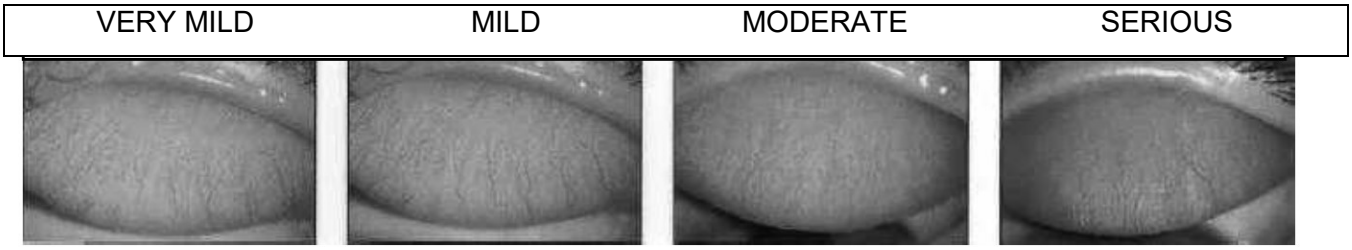



Fig. 10.3.3.2[28]

*10.3.4 Anterior segment:*

- Fotomotor and consensual pupillary reflexes, as well as the constitution of the iris will be reviewed, highlighting the pathological aspects, as inflammatory membranes, anterior or posterior synechiae, among others.
- The amplitude of the anterior chamber will be qualitatively graded by amplitude degrees with the Van Herick classification, which gives a degree by subjective assessment of the space between the iris and the posterior surface of the cornea by placing the slit lamp light beam in the outer third of the anterior chamber:

Example: Van Herick II:

- Lens: points to evaluate are: the integrity of the anterior lens capsule and the characteristics of the composition thereof, highlighting only pathological aspects, e.g. pseudoexfoliation, pigment, synechiae, etc.

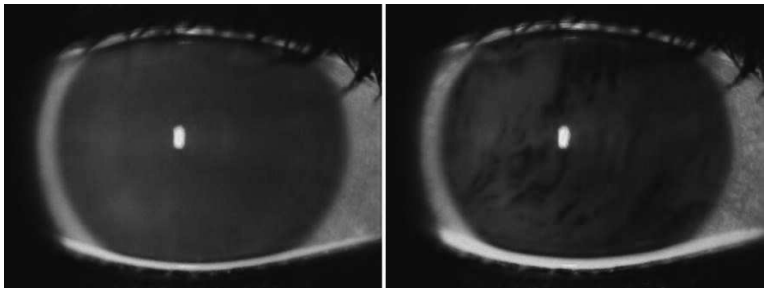
### *10.3.5 Fluorescein Staining*

Sodium fluorescein staining will be used on the ocular surface (bulbar conjunctiva and cornea) after the instillation of tetracaine for dilution. Once the surface is stained the following exploration will be performed:

### *10.3.6 Tear Drop Break-up Time*

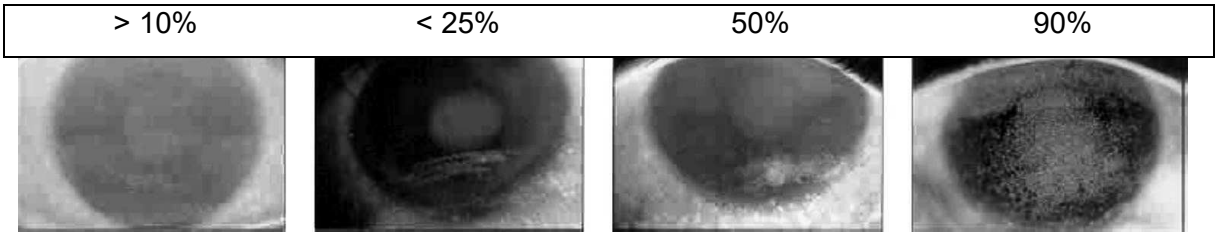
It will be measured at the end of a blink and the patient will be asked not to blink immediately until the tear film on the cornea is broken, if it exceeds 10 " it is considered as normal.

Example:

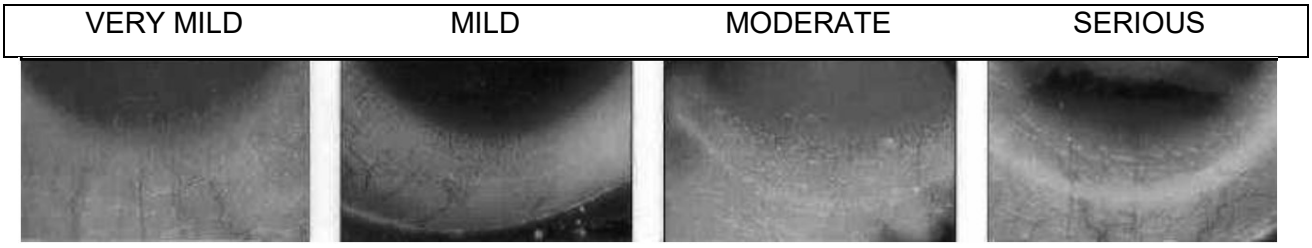


*10.3.7 Epithelial Defects on:*

10.3.7.1 CORNEA: Epithelial defects will be analized by the approximate percentage of the total corneal area, with 100% as all the corneal area.[28] Example:

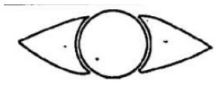



*10.3.8 CONJUNTIVA:* It will be qualitatively assessed, according to the extension of the conjunctiva fluorescein deposits, as shown below:[28]




After the fluorescein staining described is done we will take the intraocular pressure (IOP) with a Goldman tonometer, the result will be reported in millimeters of mercury, this is performed only at baseline and final visits.

Once the above aspects are valued, integrate them into a single criterion according to the scale of the University of Oxford, based on this evidence, which proves to have more correlation with the score of the OSDI and Schirmer test.

| Panel | Grade | Criterion |
|---|---|---|
| A  | 0 | Equal to or less than panel A |

| | | |
|---|---|---|
| B  | I | Equal to or less than panel B |
| C  | II | Equal to or less than panel C, more than panel B |
| D  | III | Equal to or less than panel D, more than panel C |
| E  | IV | Equal to or less than panel E, more than panel D |
| > E | V | More than panel E |

Fig. 10.3.8.1

*10.3.9 Schirmer Test:*

Schirmer test[29] involves the quantitative measurement of the amount of tears produced in the eye for 5 minutes, and it is quantified millimetrically on filter paper strips validated for the test. The procedure will be standardized as follows:

1. After instillation of topical tetracaine is instillated over the ocular surface,[29] filter paper strips are placed on the lower lid of the subjects and they will be asked to close their eyes for 5 minutes.
2. After 5 minutes the strips will be removed; the findings will be reported in millimeters.[29]
3. Likewise, assessment will be made as follows:

a Normal: > 15 mm

b Mild: < 14 mm - > 9 mm

c Moderate: > 4 mm. < 80 mm

d Serious: < 4 mm.

*10.4 Conjunctival Impression Cytology*

Impression cytology of the bulbar conjunctiva is a procedure in which the quantity and quality of epithelial cells and component of the ocular surface are evaluated; the primary focus is to evaluate the density and amount of goblet cells in the sample. The observation will take place in the single and specialized center of pathology under digital counting and it will be confirmed by observation.

The procedure consists of the following steps:

1. Tetracaine instillation over the ocular surface
2. Eye speculum placing
3. A minicell will be taken with the mounted Millipore paper.
4. This will be placed manually in contact with the Millipore surface over the nasal bulbar conjunctiva, slightly pressing, without indenting the surface.
5. Cytological fixation spray will be applied (96% alcohol cytospray)
6. This will be placed on a clean and dry bottle to transport it to the sample gathering unit

*10.5 Posterior Segment Examination:*

A review of the posterior segment shall be performed to verify that there is no pathological process that requires attention and/or prevents the entry of the subject to the trial.

The posterior segment will be explored under drug induced mydriasis, as well as the rest of the retina, checking the following details:

1. Vitreous humor appearance
2. Totally attached retina
3. Vascular alterations
4. Clinical macular area appearance
5. Foveolar area appearance
6. Optical nerve characteristics
    a. Vessel emergency
    b. Neuro-retinal ring
    c. Relation with the whole disc area and cup excavation will be expressed in decimals
7. Report any other clinical finding on the retina.

# 11. Procedures to Perform per Visit

*11.1 Baseline visit:*

1. During the visit, first the informed consent will be checked, clarifying all doubts that the subject wants to review, and recording in the case report format (CRF) the possible questions and the answers given to the subject; this procedure will be carried out strictly by the health staff, preferably by principal investigator.
2. Then, the signing of the informed consent, together with the two witnesses (if applicable) will be done.
3. Eligibility and exclusion criteria will be evaluated.
4. Pregnancy test will be performed (if it applies).
5. The medical staff will provide a patient with a medical history , emphasizing the signs and symptoms related with the systemic inflammatory diseases.
6. A complete ophthalmic clinical history, covering at least the points required on the CRF plus the points required by medical history first used on the research center will be made.
7. An evaluation about all the concomitant drugs will be done.
8. An OSDI questionnaire will be given.
9. A subject number will be given.
10. Ophthalmic examination:
    a. Visual acuity and best-corrected visual acuity
    b. Biomicroscopy
    c. Rose Bengal staining on the ocular surface
    d. Fluorescein staining on the ocular surface
    e. Intraocular pressure measuring with Goldman tonometer
    f. Schirmer Test

g. Drug induced mydriasis with tropicamide/phenylephrine.Fundoscopy on a slit lamp with a 90D or 78D fundus lens.
h. Drug product delivery
i. Impression cytology[21]

11. Delivery of the assigned medication to the subject.
12. Schedule the following visits.

### *11.2 Visit 1 (day 7)*

1. Medical and ophthalmologic clinical history.
2. Ophthalmic Examination:
   a) Visual acuity and best-corrected visual acuity
   b) Schirmer test type 2 (after tetracaine instillation 5mg/ml)
   c) Biomicroscopy (evaluate chemosis, conjunctival hyperemia, iris and crystalline appearance).
   d) OSDI questionnaire
   e) Rose Bengal staining on the ocular surface
   f) Fluorescein staining on the ocular surface
   g) Tear film breakup time.TBUT
   h) Intraocular pressure measuring with Goldman tonometer
   i) Review of attachment to medication.

3. Subject's journal checking and adherence to the study

4. Adverse events review or concomitant drug product modification
5. Schedule the following visit
6.

### *11.3 Visit 2 (day 30)*

1. Ophthalmic Examination:
    a) Visual acuity and best-corrected visual acuity measuring
    b) Biomicroscopy
    c) OSDI questionnaire
    d) Rose Bengal staining on the ocular surface
    e) Fluorescein staining on the ocular surface
    f) Tear film breakup time.TBUT
    g) Intraocular pressure measuring with Goldman tonometer
    h) Review of attachment to medication.
2. Subject's journal review and adherence to the study
3. Adverse events review or concomitant drug product modification
4. Schedule next visit.

### *11.4 Visit 3 (day 60)*

1. Medical and ophthalmologic clinical history.
2. Ophthalmic Examination:

a) Visual acuity and best-corrected visual acuity

b) Schirmer test type 2 (after tetracaine instillation 5mg/ml)

c) Biomicroscopy (evaluate chemosis, conjunctival hyperemia, iris and crystalline appearance).

d) OSDI questionnaire

e) Rose Bengal staining on the ocular surface

f) Fluorescein staining on the ocular surface

g) Tear film breakup time.TBUT

h) Intraocular pressure measuring with Goldman tonometer

i) Review of attachment to medication.

3. Subject's journal checking and adherence to the study

4. Adverse events review or concomitant drug product modification

5. Schedule the following visit

*11.4 Final visit (day 90)*

1. Quality assessment questionnaire will be applied.
2. Pregnancy test performed if necessary.
3. Medical and Ophthalmic clinical history.
4. Concomitant medication evaluation.
5. OSDI questionnaire.
6. Ophtalmic Examination:

a. Visual acuity and best-corrected visual acuity measuring
b. OSDI questionnaire
c. Biomicroscopy
d. Rose Bengal staining on the ocular surface
e. Fluorescein staining on the ocular surface
f. Intraocular pressure measuring with Goldman tonometer
g. Schirmer test.
h. Drug induced mydriasis with tropicamide/phenylephrine.
i. Fundoscopy on a slit lamp with a 90D or 78D fundus lens.
j. Impression cytology.
k. Ask for the return of the drug product given on the baseline visit.
l. Schedule the safety phone call.

7. Subject's journal review and adherence to the study.

8. Adverse events review or concomitant drug product modification.

### *11.5 Follow up phone call (day 105)*

1. Investigate about possible adverse events related with the use of the drug.

# 12. Study Population Characteristics

- Adults of ≥ 18 and ≤ 90 year old
- Both sexes
- Mild to moderate tear film dysfunction clinical diagnose
- 6 months recruiting
- Recruiting potential: once the research centers are opened it is estimated that, during the first month, inclusions will cover 75% of the total sample size.

## 12.1 Inclusion Criteria

- ≥18 to ≤ 90 years old
- Both sexes
- Mild to moderate tear film dysfunction clinical diagnose
- Mild to moderate clinical stage of the disease
  - TBUT > 5 sec. and < 10 sec.
  - Schirmer: > 4 mm and < 14 mm
  - OSDI < 30 points
  - Corneal staining < grade III on the Oxford scale
- Availability to go to each revision when indicated.

## 12.2 Exclusion Criteria

### *12.2.1 General Criteria:*

1. Subjects with topical and/or systemic medication or mechanical devices that interfere determinedly on the results of the study (such as topical immunomodulators, punctal plugs, corticosteroids, preservative artificial tears, contact lenses).
2. Subjects (females) with active sexual life that do not use a contraceptive method.
3. Female subjects who are pregnant or lactating
4. Female subjects with a positive urine pregnancy test
5. Positive drug addictions* (verbal interrogatory)
6. Subjects who have participated on any other research clinical trials on the last 40 days
7. Subjects legal or mentally disabled to give an informed consent for participating on this study
8. Subjects who can't comply with the appointments or with every protocol requirement.

*Definition: repeated ingest of one or many psychoactive substances, until the consumer (named addict) is periodically or continuously intoxicated, showing a compulsive desire of consuming the preferred substance (or substances); he/she has a big difficulty to voluntarily interrupt or modify the substance consumption and is decided to obtain it anyhow. – Definition from the WHO*

### *12.2.2 Criteria related with ophthalmic ailments*

1. Serious tear film dysfunction syndrome
    - TBUT < 5 s
    - Schrimer: < 4 mm

- OSDI > 30 pints
- Corneal staining > grade III on the Oxford scale

2. Non perforated corneal ulcer
3. Perforated corneal ulcer
4. Autoimmune corneal ulcer
5. Ocular surface scarring diseases
6. Ocular surface or annexes metaplastic lesions
7. Fibro vascular proliferation lesions on the conjunctival and/or corneal surface (i.e.: pterygium)
8. Concomitant chronic inflammatory diseases on any ocular structure
9. Acute or infectious inflammatory disease
10. Corneal disease potentially requiring a treatment during the following 3 months
11. Use of topical or systemic drug products classified as forbidden
12. Ocular surgical procedures 3 months before the protocol inclusion
13. Treatments or procedures indicated on the tear film dysfunction treatment, as punctal silicone plugs.
14. Posterior segment diseases requiring a treatment or threating the visual prognosis
15. Retinal diseases potentially requiring treatment during the following 3 months
16. History of penetrating keratoplasty.
17. Soft or hard contact lenses use during the last month from inclusion day

### *12.2.3 Elimination criteria for the subject included on the study*

1. Signs and symptoms evolving to serious tear film dysfunction syndrome detected on any treatment visit.
    a. TBUT < 5 seconds
    b. Peripheral or secondary leucoma to corneal scarring
    c. Phlycten
    d. Corneal keratinization
    e. Epithelial squamous metaplasia
    f. Corneal conjunctivalization
    g. Corneal neovascularization
    h. Corneal ulcers
    i. Any secondary injury permanently reducing the visual acuity, as a consequence of the tear film dysfunction.
2. Added ocular surface diseases requiring a different treatment to PRO-087 or added treatments indicated for a new diagnose.
3. Opacities on ocular clear media (cornea, lens, vitreous body) avoiding to assess the anterior chamber or the posterior segment.
4. Decision of the subject
5. Difficulty to attend the follow-up appointments.
6. Added or self-prescribed non-authorized treatments
7. Herbal treatments affecting the course of the disease or producing other complications not related with the disease
8. Systemic diseases requiring an immunomodulator, immunosuppressing or biological treatments affecting any inflammatory cascade line.

9. Retinal diseases secondary to chronical-degenerating diseases requiring treatment to preserve the function and/or anatomy
10. Inflammatory retinal diseases
11. Uveitis presentation installation at any ocular uveal level
12. Clinical picture meaning an ophthalmic urgency requiring immediate treatment
13. Ocular diseases requiring surgical treatment
14. Ocular diseases requiring intraocular injections

### *12.3 Forbidden medications:*

| Drug product group | Prototype | Route of administration | Washout period |
|---|---|---|---|
| *Non steroid anti-inflammatory drugs* | Ketorolac<br>Bromfenac<br>Diclofenac | Topical | 2 weeks |
| *Steroid anti-inflammatory drugs* | Dexamethasone<br>Prednisolone<br>Fluorometholone | Topical and systemic | 3 weeks |
| *Prostaglandin analogues* | Latanoprost<br>Travaprost | Topical | 4 weeks |
| *Immunomodulators* | Cyclosporine | Topical and systemic | 2 months |
| *Vasoconstrictors* | Nafazoline | Topical | 2 weeks |
| *Agonists alpha 2* | Brimonidine | Topical | 3 weeks |
| *Betablockers* | Timolol, betaxolol | Topical | 2 weeks |
| *Preservative lubricants* | Hirpomelose<br>Sodium hyaluronate<br>Propinylglycol | Topical | 2 weeks |

# 13. Sample size determination

The sample size was calculated according to the 3 major safety variables. The variable is considered the largest number of subjects obtained as the sample size required to meet the objectives of the study.

## *13.1 TBUT*

The sample size was calculated according to the formula for continuous quantitative variables:

$$n=2\left[\frac{(Z_{\alpha}-Z_{1-\beta})(\delta)}{d}\right]^{2}$$

With a statistical confidence level of 95%, which corresponds to error type I, and is equal to 1.96, with a potency of 80% corresponding to error type II, and is equal to 0.84, a standard deviation for an increase in TBUT of 1.1 seconds[22] was considered, with an expected difference of at least 0.15 seconds.

Based on the above, the result was 32 patients per group, which increased 20% in consideration of the losses, with a total of 39 patients per group, with a total study population of 116 subjects (232 eyes).

## *13.2 OSDI*

The sample size was calculated according to the formula for continuous quantitative variables:

With a statistical confidence level of 95%, which corresponds to the error type I, and is equal to 1.96, with a potency of 80% corresponding to the error type II, and is equal to 0.84, a standard deviation for the 6.4 points[23] increase in score OSDI, with an expected difference of at least 6.1 points, was considered.

Based on the above, the result was 34 patients, which increased 20% in consideration of the losses, with a total of 41 patients per group, with total study population of 123 subjects (246 eyes).

### *13.3 Schirmer*

The sample size was calculated according to the formula for continuous quantitative variables:

With a statistical confidence level of 95%, which corresponds to the error type I, and is equal to 1.96, with a potency of 80% corresponding to the error type II, and is equal to 0.84, a standard deviation for the 1.72 mm/5 $min^1$ increase in the Schirmer test, with an expected difference of at least 0.13 mm/5 min, was considered.

Based on the above, the result was 80 patients, which increased 20% in consideration of the losses, with a total of 96 patients per group, with total study population of 288 subjects (576 eyes).

Based on the above calculations and considering the primary outcome variable, and Schirmer test used in the study to demonstrate efficacy between the 3 groups, the total population to evaluate will be of 288 subjects.

## 14. Study Variables

### *14.1 Efficacy Evaluation*

The variables of the study drug PRO-087 tolerability will be evaluated in the visits indicated in the schedule, using the following parameters:

1. Best-corrected visual acuity
2. Corneal depithelization
3. Tear film break-up time

4. Schirmer test
5. OSDI
6. Goblet cells population

It is considered that the drug in evaluation PRO-087 is effective for use in patients diagnosed with mild to moderate tear film dysfunction, if they exhibit the changes marked in parameters (14.1.2) by more than 5% of subjects in both eyes, in compliance with all visits and adherence to treatment at an 80% during the time of study assessment.

### *14.1.2 Efficacy Assessment Parameters*

| Parameters | Value |
|---|---|
| **Best-corrected visual acuity** | Increase of 1 line in the Snellen chart or 1 logarithmic point (logMAR) |
| **Corneal depithelization** | Percentage of the damaged epithelium of the ocular surface, reduction of staining according to the oxford scale. |
| **Tear film break-up time** | Increase in the residence of the teal film, measured in seconds. Increase of 30% from baseline or equal or more than 10 sec. |
| **Schirmer test** | Increase of more than 30% |
| **Adverse events** | Presence of adverse events modifying some of the abovementioned criteria or others, evaluated as serious. |
| **OSDI** | Reduction of 20% of the total score from the baseline score. |
| **Goblet cells population** | Increase of 20% from baseline |

## *14.2 Tolerability Evaluation*

Tolerability variables of the study drug PRO-087 will be evaluated in the visits indicated in the schedule, using the following parameters:

1. Burning
2. Foreign body sensation

3. Tearing
4. Conjunctival hyperemia
5. Pruritus
6. Photophobia
7. Tear secretion

It is considered that the drug in evaluation PRO-087 is tolerable for use in human beings, if there are no changes on the marked parameters (14.1.2) by more than 5% of subjects, in both eyes, in compliance with all visits and adherence to treatment at an ≥ 80% during the time of study assessment.

*14.2.1 Tolerability Parameters*

| Parameters | Value |
|---|---|
| **Burning** | It should be recorded as absent on 95% of the evaluated subjects |
| **Foreign body sensation** | It should be recorded as absent on 95% of the evaluated subjects |
| **Tearing** | It should be recorded as absent on 95% of the evaluated subjects |
| **Secretion** | It should be recorded as absent on 95% of the evaluated subjects |
| **Conjunctival hyperemia** | The following must be accomplished:<br>• Absent: ≥ 95.0%<br>• Mild:≤ 5.0%<br>• Moderate: ≤ 2.5%<br>• Serious: ≤ 1.0% |
| **Photophobia** | The following must be accomplished:<br>• Absent: ≥ 95.0%<br>• Mild: ≤ 5.0%<br>• Moderate: ≤ 2.5%<br>• Serious: ≤ 1.0% |

Safety variables of the study drug PRO-087 will be evaluated in the visits indicated in the schedule, using the following parameters:

1. Best-corrected visual acuity
2. Intraocular pressure
3. Ocular surface
4. Anterior segment exploration
5. Posterior segment exploration
6. Tear film break-up time
7. Schirmer test
8. Corneal depithelization
9. Adverse events

It is considered that the drug in evaluation PRO-087 is tolerable for use in human beings, if there are not changes on the marked parameters (14.3.1) by more than 5% of subjects, in both eyes, in compliance with all visits and adherence to treatment at an ≥80% during the time of study assessment.

### *14.3.1 Safety Parameters*

| Parameters | Value |
|---|---|
| **Best-corrected visual acuity** | No change of 1 line in the Snellen chart or 1 logarithmic point (logMAR) |
| **Intraocular pressure** | Measurements below 7 mmHg and over 21 mmHg |
| **Corneal depithelization** | Percentage of the damaged epithelium of the ocular surface, reduction of staining according to the Oxford scale. |
| **Anterior segment examination** | Abnormalities on any of the comprised structures: Cornea, iris, pupil, iris-corneal angle and crystalline. |
| **Posterior segment examination** | Abnormalities on any of the comprised structures: Vitreous, retina, macula and optic nerve. |
| **Tear film break-up time** | Reduction of more than 20% over the baseline time. |
| **Schirmer test** | Reduction of more than 20% |
| **Adverse events** | Presence of adverse events modifying some of the abovementioned criteria or others evaluated as serious. |
| **OSDI** | Increase of the score of more than 10 points. |

# 15. Proposed statistical analysis.

The statistical analysis will be performed by an investigator blinded to the intervention groups.

The continuous quantitative variables will be presented by measures of central tendency and dispersion (mean, standard deviation and ranges). Nominal and ordinal qualitative variables will be introduced by means of frequencies and ratios.

The Kolmogorov-Smirnov test will be performed to know the normal or abnormal distribution of the results obtained in each study group.

Assuming there is a normal distribution of data, the intra-group differences will be determined by an ANOVA test for repeated measures, with the Pearson correlation coefficient, for the quantitative variables.

For qualitative variables the $\chi^{2^2}$ (chi square) test will be used and in case the expected frequencies are less than 5 the Fisher's exact test will be used.

The differences between groups will be analyzed using the ANOVA test for variance analysis, with the Pearson correlation coefficient, for the qualitative variables.

For qualitative variables, contingency tables 2x2 will be used and the differences will be calculated with $\chi^{2^2}$ (chi square) with Yates's correction and in case the expected frequencies are less than 5 the Fisher's exact test will be used.

The significance level will be of a 0.05 alpha value or less.

The final statistical analysis will include all the subjects that complete the visits listed in the activity chronogram.

Also, those who have been assigned with a treatment (intervention) and attend at least to 1 visit after the basaline will be included and analyzed until the moment they attended.

Those who do not attend at least 1 follow up visit will be excluded.

Those who do not complete the visits will be classified as population with intention to treat.

# 16. Results reporting.

The results obtained during the study protocol will be the sponsor's responsibility, who has the obligation to report the results to the competent authorities. Also, each one of the participating investigators will receive a report of the results.

In order to disseminate the obtained scientific knowledge, the publication of the study in an international magazine will be procured.

# 17. Identification, safety and therapeutic compliance procedures of the research subjects.

### *17.1 Recruitment*

Patients will be recruited at the ophthalmology practice of the participant Contract Research Organization and a goal of 20 scrutinized and randomized patients is contemplated.

### *17.2 Patient's identification*

All the participants of the study will be identified with a number and the initials of their name.

In the scrutiny stage, the participant number will be assigned consecutively, using 3 digits and in the enrollment stage a number will be assigned according to an electronic randomization system. Once the subject is included a patient number will be assigned and it will be composed of 3 molecule digits, 2 of the center number and 3 digits of the assigned consecutive number in the investigation center.

Example:

**080701001** (molecule number/center number/patient number in the center).

### *17.4 Patient elimination*

The causes for a withdrawal of the study from a patient might be the following:

Subject's decision. The patient who wishes to leave the study for any reason can do so at any time, but the investigator must be informed of this. In all cases, the investigator must try to get in contact with the patient as soon as possible for a final assessment in order to do the following:

Document the patient's decision in clinical notes,

Obtain the reason(s) of the departure and record them in the departure form of the CRF,

Assess the clinical state of the patient,

If necessary, take the appropriate therapeutic measures: management of an adverse event or concomitant disease.

Investigator's decision: Especially if there is an adverse event and if the investigator considers that this might threaten the patient's health, actions will be taken as described on the adverse events section or if there is an important disease that needs the prescription of a drug incompatible with the purpose of the study. It will be reported in clinical notes and a serious adverse events report format will be filled and the Research Ethics Committee, the clinical monitor and Pharmacovigilance of Laboratorios Sophia will be informed of this.

A wrongful inclusion according to the protocol. The decision to keep the patient in the will be taken conjointly by the investigator and the sponsor.

Other reasons (insufficient response, need for another treatment).
In all cases, the available data will be kept for safety analysis (assessable population for intention to treat).

### *17.5 Investigational products recall*

In case of an investigational product recall (decided by the competent authorities or by the sponsor), the sponsor will inform the principal investigator immediately.

The investigator, along with the sponsor's representatives (clinical monitor) must urgently do the following:

- Stop the patient's supply of the affected investigational products.
- Inform the involved patients to immediately suspend the administration of those investigational products and take them to the center.

- ❖ The monitor will organize the return to proceed to destroy the study medication, both the used and the non-used ones for Laboratorios Sophia, as per their procedures.

### *17.6 Treatment compliance*

The study drug administration compliance from the patients will be assessed in the Laboratorios Sophia facilities once the dropper bottles of each patient are returned by the investigator; each bottle will be weighed in an analytical balance and the difference will be obtained regarding the initial weight of the container, all values over 80% will be considered as appropriate compliance to the treatment.

In case the study drug compliance is less than 80%, the obtained results will be considered to be analyzed via the intention to treat, see statistical analysis section.

# 18. ADVERSE EVENTS.

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### *18.1 Adverse event, definition and communication*

#### *18.1.1 General aspects*

During the study, adverse events (AE) may occur, these can be associated or not with the study drug.

If at any moment any of the study patients would have any adverse event classified as serious deriving from the administration of the drug, actions will be taken as per the guidelines of Official Mexican Standard 220 SSA1 2012 Installation and operation of Pharmacovigilance. The administration of the drug will be interrupted and the administration of an alternative therapy will be ordered, at the discretion of the principal investigator.

An adverse event is any adverse medical occurrence in a clinical research patient or subject to whom a pharmaceutical product has been administered and that does not necessarily have a causal relationship with this treatment. Therefore, an AE might be any unfavorable and non-intentional sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a (investigational) drug product whether it is related or not.

The investigator must report the adverse events occurred during the realization of the study from the moment a patient signs the informed consent to be included in the clinical study until the last procedure described in the approved study protocol version has ended.

In all cases, the etiology must be identified as far as possible and notify the study sponsor. An "unexpected adverse event" is any AE that is not identified in nature, seriousness or frequency in the investigator's brochure or in the risks information described in this protocol.

### *18.2 Classification and severity*

#### *18.2.1 Classification*

Adverse events, according to their importance, are divided in two categories: "serious" and "non-serious". This classification determines the procedure to be used to report or document the adverse event.

A **serious adverse event** is that which:

- Endangers or causes the patient's death.
- Makes it necessary to be hospitalized or extend the hospital stay.
- It is a motive for persistent or significant disability.
- It is a motive for alterations or malformations in the newborn.

A non-serious adverse event is that which does not fulfill the aforementioned criteria.

### *18.2.2 Adverse events severity*

The severity degree of the adverse events must be assessed by the investigator in charge or the person in charge of that function, as per the intensity of the clinical manifestations.

- **Mild severity** is considered for those adverse events that do NOT interfere with everyday activities, they may or may not require the drug suspension.
- **Moderate severity** is considered for those adverse events that interfere with everyday activities (they might cause work or school absence) without directly threatening the patient's life, they may or may not require the drug suspension.
- **Serious severity** is considered for those adverse events that prevent someone from doing everyday activities.

### *18.3 Non-serious adverse events report*

Reports of adverse events classified as "non-serious" must be made in the specific section of the case report form specific of the clinical protocol, as per the statements in the case report form completion guide.

During each monitoring visit the right completion of the non-serious adverse event report will be verified in the specific section of the case report form. It must be ensured that, before the base closure of the study, the non-serious adverse events are completed with the adverse events final resolution and the entirety of the requested information in the case report form.

### *18.4 Adverse events report characteristics*

The investigator in charge and/or the person in charge of said function must report all serious or non-serious adverse events occurring during the study development, granting a diagnosis (name) to the condition(s) seen in the patient.

It must be ensured that the adverse events are reported correctly grouping the signs and symptoms of the patient in clinical diagnosis.

In those cases where the presence of various adverse events is detected at once and that there is evidence of none of them corresponding to the same pathological entity, each one of the adverse events must be reported separately.

For the scheduled surgical interventions a clinical diagnosis that was the cause to do the surgery must be reported and not the surgical event itself. For example: cholecystectomy (surgical event-NOT reported as an adverse event), acute cholecystitis (clinical diagnosis-reported as an adverse event).

The adverse events reports must state the date/time of start, date/hour of termination, evolution and actions taken according to the following:

- Resolved.
- Resolved with sequelae.
- Being resolved.
- Not resolved.
- Unknown.

Information on adverse events must be updated, stating the date of the type of final resolution of the adverse event or record them as “being solved” for those cases when the adverse event is still active.

### *18.5 Causality*

Based on the physical examination, signs and symptoms mentioned by the study subject, the principal investigator must state, on clinical judgement, if there is or not a causal relationship between the study drug(s) and the adverse event seen in the patient.

The way to classify the causal relationship with the study drug will be the following:

***18.5.1 Definitively related:*** There is certainty that the adverse event is related to the investigational product.

***18.5.2 Probably related:*** There is a strong likelihood that the adverse event is related to the investigational product.

***18.5.3 Possibly related:*** There is a possibility of the investigational product being the cause of the adverse event, but other causes cannot be discarded.

***18.5.4 Not related:*** There is evidence suggesting the adverse event is related to a cause different to the investigational product.

***18.5.5 Unknown:*** Adverse events for which causality evaluations cannot be made.

Relationship between the study drug with the adverse events is stablished according to the following criteria:

- Previous experience with the drug.
- Knowledge of the adverse reactions with its use.
- Explanations for adverse reactions.

- ❖ Use of other drugs, concomitant diseases, non-pharmacological therapies, diagnostic tests or other procedures or, in its case, confounding factors.
- ❖ Time elapsed between the drug administration and the adverse event.
- ❖ Drug concentrations.
- ❖ Effects with re-exposure to the drug.

#### *18.5.6 Reporting of serious adverse events*

An active search of SAEs must be carried out using the spontaneous reports from the patient, the physical examinations and the interviews carried out by the investigator during the various visits.

If the AE leads to the interruption of the trial, the investigator must complete the CRF, in the corresponding section to the termination of the trial and specify if the event is serious or not.

A serious adverse events form must be filled by the principal investigator or the doctor in charge and it must be sent to the sponsor within 24 hours of the notification; even if all the information required is not available at that moment. All the subsequently gathered information must be sent immediately once it is available. The investigator must monitor the SAE properly and in its entirety. In any moment an updated report of the SAE must be given and a written report must be elaborate when the SAE reaches a final result.

Also, the Research Ethics Committees must be informed of the SAEs as well as the regulating authorities.

There must be a notification on all the SAEs as soon as they become known, according to the aforementioned definitions and regardless of the treatment or relationship to the drug being investigated.

The investigator must notify the sponsor about the event sending said notification within 24 hours after the event has become known, the “Serious Adverse Event Report” form (“first notification”) must be sent via **e-mail** with all the available information on the serious adverse event to the Pharmacosurveillance Department of Laboratorios Sophia, S.A. de C.V. and attach a copy of the e-mail to the monitoring person in charge of the center.

Person in charge of Pharmacovigillance:

**Dr. Alicia Paulina Melgarejo Martin Anaya**

E-mail: farmacovigilancia@sophia.com.mx

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Direct line, toll free number: 01 800 7102 254

The non-serious adverse events (mild and moderate) must be reported to the regulating authority at the end of the trial (after the report or in it) and the serious ones must be reported immediately.

### *18.6 Follow-up*

The investigator must prepare a specific clinical report and use the serious adverse event report and collect the results of the performed examinations and the hospitalization reports.

Serious adverse events must be followed until their resolution or stabilization.

#### *18.6.1 Serious adverse events occurring after the trial*

The sponsor must be notified of all the SAEs occurring within 15 days after the last administration of the study drug or after the end of the trial for the subject.

The sponsor must also be notified of any event occurring in any moment after the end of the trial and that might be related to the study treatment in the opinion of the investigator.

## 19. Monitoring and auditing procedures.

Each research center and in turn, each investigator will have an assigned person in charge of clinical monitoring authorized to check, surveil and search for the obtained information of the subjects in the trial.

Each research center will be submitted to an evaluation before being included to grade its human, technologic and logistic resources and its viability to perform the trial will be determined.

Periodical monitoring visits will be scheduled for the revision of files and the extraction of the required information in the case report forms (CRF); this will be performed physically in paper in the clinical file and electronically in the forms located in the electronic information site on line.

The investigators and their coordinators will have access to this site in order to perform said procedure within 72 hours after the date of the visit of the subject so that the faithfulness of the obtained data is kept and to be able to detect a possible discrepancy of any kind and in doing so, being able to perform actions more opportunely.

Audits will be performed by an external provider and the research center in which the visit will take place will be previously notified. The result of the audit will be evaluated by the Clinical Research Department of Laboratorios Sophia along with the auditors and the principal investigator. The findings will be described in a corrective and preventive actions report (CAPA) which will be filed in the general folder of the clinical trial of the department. The detected findings in the audit will be promptly followed up until their conclusion.

Audit-Inspection-Verification

The investigator must be informed about the possibility of an audit being performed during or after the study is over.

The investigator must be informed that the competent authorities may also perform an inspection or verification of the sponsor facilities and the center(s) of the study. The sponsor will inform the corresponding investigators immediately once the notification of an inspection is received in the study centers. Likewise, the investigator will inform the sponsor about any pending inspection.

The investigator will allow the representatives of the competent authorities and the people in charge of the audit to do the following:

- To inspect the site, the facilities and the material used for the trial,
- To reunite with all of its team members participating in the study,
- To have direct access to the data from the study and to the source documents,

- To consult all documents regarding the trial.

If computerized medical files are used, the investigator agrees to provide all the source documents and printings of the medical files of the participants and, if the computing system allows it, to register the changes performed during the trial.

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# 21. APPENDICES

Appendix 1: World Medical Association Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical principles for medical research involving human subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and amended by the:

29th WMA General Assembly, Tokyo, Japan. October 1975

35th WMA General Assembly, Venice, Italy, October 1983

41st WMA General Assembly, Hong Kong, September 1989

48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996

52nd WMA General Assembly, Edinburgh, Scotland, October 2000

53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)

55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)

59th WMA General Assembly, Seoul, October 2008

64th WMA General Assembly, Fortaleza Brasil, October 2013

***Introduction***

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects**,** including research on identifiable human material and data.

   The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.

2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.

3. The Declaration of Geneva of the WMA binds the physician with the words “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient’s best interest when providing medical healthcare”.

4. It is the duty of physicians to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician’s knowledge and conscience are dedicated to the fulfillment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and individual rights.

8. Even though the main objective of medical research is to generate new knowledge, this objective shall never overweigh the rights and interests of the individual taking part in this research.

9. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility of the protection of the individuals taking part in research shall always rely on a physician or any other health professional and never on the participants, even if their consent has been granted.

10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable current international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
12. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific and ethical training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.
13. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
14. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.
15. Appropriate compensation and treatment shall be guaranteed for the individuals harmed during their participation in the research.

    Risks, burdens and benefits
16. In medical practice and in medical research, most interventions involve risks and burdens.

    Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
17. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.

    Measures must be taken in order to reduce the risks to a minimum. Risks shall be continually monitored, assessed and documented by the investigator.
18. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed.

When the implied risks are more important than the expected benefits or if there is conclusive proof of definitive results, physicians shall assess if they continue, modify or immediately suspend the study.

Vulnerable individuals and populations

19. Some groups and individuals subjected to research are particularly vulnerable and might have more possibilities to suffer abuse or additional harm.

    All the vulnerable groups and individuals shall receive specific protection.

20. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population. Also, this group might stand to benefit from the knowledge, practices or interventions derived from the research.

***Scientific requirements and investigation protocols***

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
22. The design and performance of each research study involving human subjects must be clearly described in a research protocol.

    The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

    In clinical studies, the protocol should include arrangements for post-study provisions.

***Research ethics committees***

23. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the investigator, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The investigator must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee. After a study has ended, the investigators shall submit a final report to the committee with a summary of the results and conclusions from the study.

***Privacy and confidentiality***

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

***Informed consent***

25. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the investigator, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After assuring that the subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject’s freely-given informed consent.

If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.
All the individuals participating in medical research must have the option to be informed about the general results of the study.

27. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.

31. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.

32. For medical research using identifiable human material or data such as research on material or data contained in biobanks or similar deposits, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:

The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists or

Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention less effective than the best proven one, the use of placebo or no intervention whatsoever.

Patients who receive an intervention less effective than the best proven one, placebo or no treatment will not be subject to additional risk of serious or irreversible harm as a consequence of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

***Post study provision***

34. Before the clinical study, the sponsors, investigators and the governments of the host countries must provide the post study access to all the participants who still need an intervention which is identified as beneficial in the study. This information must also be provided to the participants during the informed consent process.

Recording and publication of the research and dissemination of results

35. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.

36. Investigators, authors, sponsors, directors and editors all have ethical obligations with regard to the publication and dissemination of the results of the research.

Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

***Unproven interventions in clinical practice***

37. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

The good clinical practice directives of the International Conference on Harmonization contain 13 basic principles which are based on the Declaration of Helsinki. These are the principles.

1. The first principle states that the clinical studies must be performed according to the ethical principles of the Declaration of Helsinki. These ethical principles also match the Good Clinical Practice and the requirements of the local regulating institutions.

2. The second principles states that before carrying out a clinical study, the possible risks and inconveniences should be weighed against the benefits anticipated to be obtained for the subjects of the study and for society in general. A study can only be started and fully developed if the anticipated benefits justify the risks.

3. The third principle of Good Clinical Practice states that rights, safety and the well-being of the subjects of the study are the most important considerations and that they should prevail over the interests of society and science.

4. The fourth principle states that the information available before the study on an investigational product should be appropriate to support the proposal of performing the clinical study.

5. The fifth principle states that the clinical studies should have reasonable scientific foundations and that the studies should be clearly described and in detail in a protocol. The requirements of this protocol of the clinical study are also described in the Good Clinical Practice directives of the ICH.

6. The sixth principle states that the study must be performed according to the protocol that has been already approved by the institutional review board or the independent ethics committee. This means that a study cannot be started until the approval from these institutions is obtained.

7. The seventh principle states that the medical care for research subjects and the medical decisions taken in regards to them should be taken by a physician or a dentist, when appropriate. The people responsible for medical care should always be qualified physicians.

8. The eighth principle states that each individual participating in the clinical study should have the proper education, training and experience to participate and to perform the responsibilities assigned in the clinical study. Generally the sponsor is responsible for assuring all people working in the performance of the study are sufficiently trained to carry it out as much as for their education as for their experience and training. This assessment is made in the pre-study visit, in which the sponsor reunites with the personnel and interviews each member to make sure they each one has the proper training to perform the study. As part of this assessment, the sponsor checks the resumes (CV) of the personnel participating in the study.

9. The ninth principle states that informed consent should be obtained from each study subject before the start of the research and the participation of the subject. It is very important to point out that the voluntarily granted informed consent should be ready before the study and that possible subjects should not start to be examined with selection purposes to be a part of the study, before the subjects have voluntarily granted their informed consent.

10. The tenth principle states that all the information from the clinical study should be documented and filed so that it allows for the reports to be prepared. Also, this information should be able to be interpreted and verified. In other words, this principles states that the information delivered to the sponsor should be able to be exactly verified, reported and interpreted.

11. The eleventh principle states that the confidentiality of the data which allows the identification of study subjects. Respect for privacy and confidentiality rules should follow the corresponding regulation.

12. The twelfth principle of Good Clinical Practice states that the investigational products should be manufactured, administered and stored according to the industrial good manufacturing practices. The Good Manufacturing Practices have existed for much longer than Good Clinical Practice and many countries in the world have followed the Good Manufacturing Practices for the manufacture and storage of investigational products. Investigational products used in a clinical study should be used according to the approved protocol. In the protocol there is a section containing the detailed instructions for the manufacture, handling and storage of the investigational product in a clinical study.

13. The thirteenth principle states that procedure systems should be established in order to assure the quality of all the aspects of the clinical study. Generally this is the sponsor's responsibility.

In summary, we have seen the Good Clinical Practice directives established by the International Conference on Harmonization, we have described their historical development and also their current content. We have seen the different sections of these regulations and we have established that the glossary functions as a common language for global clinical research. We have also described the current version of the Good Clinical Practices of January 17th of 1997, which include an addenda titled, Guidance notes, of September 8th of 1997.

Document consulted in the international regulation collection of the National Commission of Bioethics: consulted at

http://www.conbioeticamexico.salud.gob.mx/interior/normatividad/normainter.html on March 12 of 2015

*The OSDI (ocular surface disease index) test is a simple test created to establish the seriousness and classification of dry eye according to their symptoms.*

*Answer the following questions by circling the box which best represents your answer:*

1. Have you experienced any of the following during last week?

| | FREQUENCY | | | | |
|---|---|---|---|---|---|
| | All of the time | Most of the time | 50% of the time | Some of the time | None of the time. |
| Eyes that are sensitive to light. | 4 | 3 | 2 | 1 | 0 |
| Eyes that feel gritty. | 4 | 3 | 2 | 1 | 0 |
| Painful or sore eyes. | 4 | 3 | 2 | 1 | 0 |
| Blurred vision. | 4 | 3 | 2 | 1 | 0 |
| Poor vision. | 4 | 3 | 2 | 1 | 0 |
| | | | | Subtotal: | |

2. Have problems with your eyes limited you in performing any of the following during last week?

| | FREQUENCY | | | | |
|---|---|---|---|---|---|
| | All of the time | Most of the time | 50% of the time | Some of the time | None of the time. |
| Reading. | 4 | 3 | 2 | 1 | 0 |
| Driving at night | 4 | 3 | 2 | 1 | 0 |
| Working with a computer or bank machine (ATM) | 4 | 3 | 2 | 1 | 0 |
| Watching TV | 4 | 3 | 2 | 1 | 0 |
| | | | | Subtotal: | |

3. Have your eyes felt uncomfortable in any of the following situations during last week?

| | FREQUENCY | | | | |
|---|---|---|---|---|---|
| | All of the time | Most of the time | 50% of the time | Some of the time | None of the time. |
| Windy conditions. | 4 | 3 | 2 | 1 | 0 |
| Places or areas with low humidity (very dry). | 4 | 3 | 2 | 1 | 0 |
| Areas that are air conditioned. | 4 | 3 | 2 | 1 | 0 |
| | | | | Subtotal: | |

OSDI score= total score × 25 / number of answered questions