Excelencia en oftálmicos

Statistical Analysis Plan

Phase IV clinical study, to compare the efficacy of the combination of Krytantek Ofteno PF® plus Gaap Ofteno PF® against the combination of Eliptic Ofteno PF® plus Gaap Ofteno PF®, in patients with primary open-angle glaucoma or ocular hypertension.

Protocol Code: SOPH122-0420/IV

Protocol Version: 2.0

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

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Content

1. Abbreviations

AVMC	Best corrected visual acuity
CRF	Electronic case report form (*case report) form*)
EA	Adverse events
EOF	Eliptic Ofteno PF® (timolol 0.5%/dorzolamide 2%)
FCI	Informed Consent Form
GCC	Central corneal thickness
GOF	GAAP Ofteno PF® (latanoprost 0.005%)
GPAA	Primary open-angle glaucoma
HTO	Ocular hypertension
ITT	Intention-to-treat population
KOF	Krytantek Ofteno PF (timolol 0.5%/dorzolamide 2%/brimonidine 0.2%)
MC	Concomitant medication
OCT	*Optical* coherence tomography
PF	Preservative - free
PI	Research Product
PIO	Intraocular pressure
PP	Population by protocol
DRC	Cup/disc ratio
TMM	Maximum medical therapy

2. Introduction

This document describes the statistical analysis plan (SAP) designed for protocol SOPH122-0420/IV (Phase IV clinical study to compare the efficacy of Krytantek Ofteno PF® in patients with primary open-angle glaucoma or ocular hypertension). This SAP is intended to supplement the study protocol. Any deviations from this document will be described in the Final Clinical Study Report [1].

3. Study Design

Phase IV, randomized, double-masked, multicenter, controlled, parallel-group clinical trial. The investigator, patient, and statistical analysis will be blinded.

The study will consist of two treatment phases, see Figure 1.

- First phase (eligibility/open phase): In this approximately 30-day phase, participants who meet the eligibility criteria (see 3.4 Selection Criteria) will be assigned to concomitant IOP-controlling therapy (Gaap Ofteno PF®, GOF). The purpose of this phase is to homogenize patients on a single treatment before exposing them to the experimental treatment.
- Second phase (double-blind phase): In this approximately 60-day phase, participants who again meet the eligibility criteria (see 3.4 Selection Criteria) will be randomly assigned to the experimental treatment (investigational treatment [Krytantek Ofteno PF®, KOF] or comparator treatment [Eliptic Ofteno PF®, EOF]), while continuing on concomitant treatment (GOF). It is in this phase that patients are exposed to maximal medical therapies (MMT).

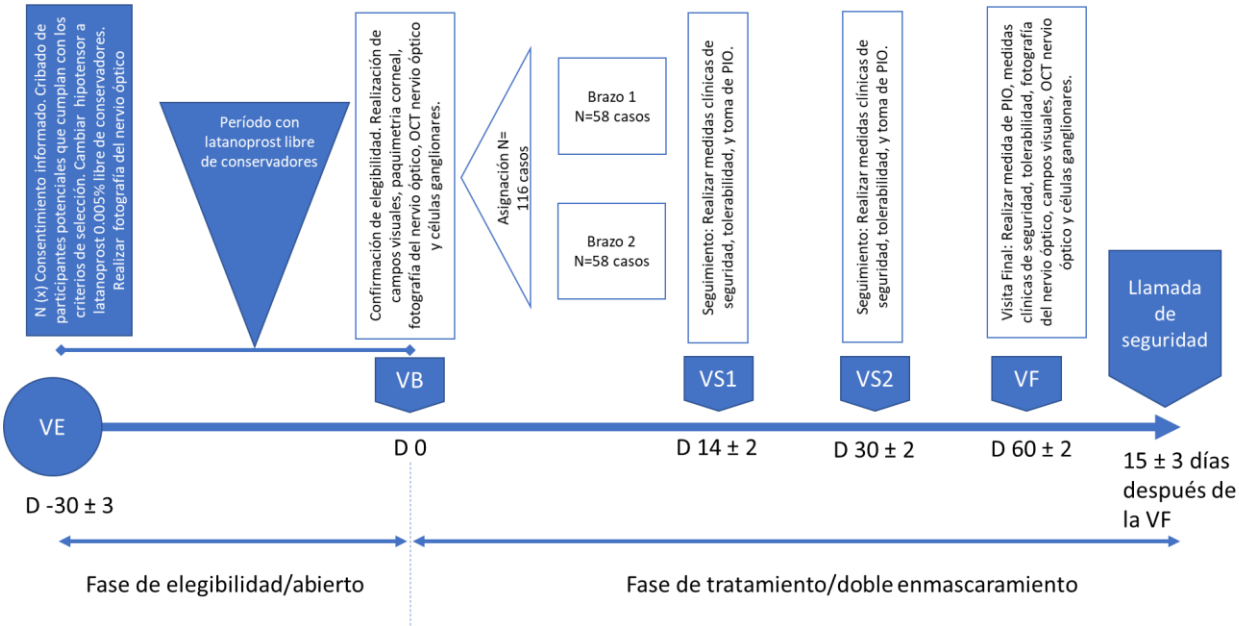

Figure 1. Design study graph

3.1 . Objectives of the Study

3.1.1 Primary objective

To determine the superiority in lowering mean intraocular pressure of the preservative-free fixed combination of timolol 0.5%/dorzolamide 2%/brimonidine 0.2% (Krytantek Ofteno PF®), compared to the effect obtained by the preservative-free fixed combination of timolol 0.5%/dorzolamide 2% (Elliptic Ofteno PF®), in subjects with primary open-angle glaucoma (POAG) or ocular hypertension (OHT) treated with preservative-free latanoprost (Gaap Ofteno PF®).

3.1.2 Secondary objectives

- To determine the efficacy of adding the preservative-free (KOF) timolol 0.5%/dorzolamide-2-rimonidine 0.2% fixed combination to patients with POAG or OHT treated with preservative-free (GOF) latanoprost.
- To determine the efficacy of adding the preservative-free (PF) timolol 0.5%/dorzolamide 2% fixed combination to patients with POAG or OHT treated with preservative-free (PFL) latanoprost.

3.2 Study Hypothesis

H_0: The mean final reduction in intraocular pressure obtained by adding the preservative-free (KOF) timolol 0.5%/dorzolamide 2%/brimonidine 0.2% fixed combination is not superior to the effect obtained by adding the preservative-free (EOF) timolol 0.5%/dorzolamide 2% fixed combination in patients with POAG or OHT treated with preservative-free (GOF) latanoprost. A difference ≥ 1.5 mmHg between treatments is considered superior.

$$H_0: \mu_A - \pi_B \leq \delta$$

H_1: The mean final reduction in intraocular pressure obtained by adding the preservative-free (KOF) timolol 0.5%/dorzolamide 2%/brimonidine 0.2% fixed combination is superior to the effect obtained by adding the preservative-free (EOF) timolol 0.5%/dorzolamide 2% fixed combination in patients with POAG or OHT treated with preservative-free (GOF) latanoprost. A difference ≥ 1.5 mmHg between treatments is considered superior.

$$H_1: \pi_A - \mu_B > \delta$$

3.3. Study Variables

3.3.1 Effectiveness Variables

- Average IOP reduction achieved with the experimental treatment when added to patients treated with GOF
- Percentage of patients treated with GOF, who when one of the experimental treatments is added, achieve a decrease of ≥20%, ≥25%, ≥30%, and ≥35% in their IOP
- Percentage of patients treated with GOF, who when adding one of the experimental treatments achieve a decrease of ≤12, ≤13, ≤14, ≤15, and ≤18 mmHg

3.3.2 Security variables

- AVMC
- Cup/disc ratio measured by the researcher and by OCT
- Nerve fiber layer and ganglion cell thickness measured by OCT

- Evaluation of the optic nerve by optic nerve photography
- Comparison of optic nerve photography at baseline and at the end of the study
- Visual fields determined by computerized campimetry
- GCC determined by pachymetry
- Integrity of the ocular surface:
  - Conjunctival hyperemia
  - Chemosis
  - Fluorescein staining
- EA

3.3.3 Tolerability Variables

- Eye comfort index (ICO)

3.4 Selection Criteria

3.4.1 Inclusion Criteria

- Subjects with POAG (according to the American Academy of Ophthalmology preferred practice pattern guidelines) or HTO, not controlled by a prostaglandin analogue or β-blocker in the eye to be included in the study.
- Treatment prior to the eligibility visit for ≥ 30 days with a prostaglandin analogue or β-blocker in the eye to be included in the study.
- IOP by Goldmann tonometry ≥ 19 and ≤ 26 mmHg in the eye to be included in the study.
- Ability to voluntarily grant informed consent.
- Be able and willing to comply with scheduled visits, treatment plan, and other study procedures.
- Age equal to or greater than 18 years.

3.4.2 Exclusion Criteria

- Being pregnant, breastfeeding, or planning to become pregnant during the clinical study.
- For women of reproductive age, not having a hormonal contraceptive method, intrauterine device, or bilateral tubal obstruction for a period of 30 days or more.
- Anterior chamber angle <2 in the Shaffer classification or presence of peripheral anterior synechiae in the eye to be included in the study.
- Being treated with any systemic hypotensive agent (e.g. mannitol, glycerin, isosorbide).
- BCVA less than 20/200 in the eye to be included in the study.
- Severe central visual field loss (sensitivity ≤ 10 dB at ≥ 2 of the 4 points central to the fixation point of the visual field), in the eye to be included in the study.
- History of ocular surgery or ocular laser procedure within the last 6 months in the eye to be included in the study.
- History of ocular trauma in the last 6 months in the eye to be included in the study.
- History of chronic uveitis in the eye to be included in the study.
- retrobulbar , subconjunctival or subtenon injection in the last 6 months in the eye to be included in the study.
- Patients with silicone, or who have had silicone, in the anterior or posterior segment of the eye to be included in the study.

- Diagnosis of aphakia in the eye to be included in the study.
- Any corneal alteration that decreases the reliability of Goldmann tonometry in the eye to be included in the study.
- Known hypersensitivity to the active ingredients to be used in the study (prostaglandin analogues, β-adrenergic blockers, α2-adrenergic agonists, IAC).
- Diseases that contraindicate the use of the active ingredients to be used in the study (e.g. severe asthma or COPD, 2nd or 3rd degree atrioventricular block not controlled with a pacemaker, sinus bradycardia, manifest heart failure, chronic kidney disease with a CrCl < 30 ml/min).
- Patients requiring the use of MAO inhibitors, and patients treated with antidepressants that affect noradrenergic transmission (e.g. tricyclic antidepressants and mianserin).
- Patients who use, or have used in the last month, topical ophthalmic steroids in the eye to be included in the study, or by the following routes: oral, intravenous, intramuscular, dermal, or intralesional .
- Patients who use, or have used in the last month, oral, intravenous, or intramuscular steroids.
- Having participated in another clinical research study ≤ 30 days prior to signing the FCI.
- Having previously participated in this study.
- Having a history of drug addiction within the last two years prior to signing the FCI.
- Have any type of surgical intervention scheduled during the study period.
- Be or have an immediate family member (e.g., spouse, parent/legal guardian, sibling, or child) who is a member of the research site or sponsor staff.

3.4.3 Elimination Criteria

- IOP ≤ 18mmHg at the baseline visit, in the eye to be included in the study.
- IOP > 26 mmHg at the baseline visit, in the eye to be included in the study.
- Subject's decision.
- Pregnancy.
- Presence of a serious EA.
- Lack of efficacy of maximum medical therapy (less than 20% decrease when any of the experimental treatments were added to the GOF treatment).
- Adherence less than 90% to the treatments provided in the study.
- Subject who does not attend two consecutive visits.

3.5 Subject's duration in the study/treatment

The duration of treatment is 90 days, and the subject's approximate duration in the study is 105 days. The procedures and visits to be performed during each phase of the study are shown in Table 1 .

Table 1. Study schedule

Procedures	Eligibility Phase		Double-masking phase			
	VE	VB	V1	V2	VF	LLS
	D -30±3	D 0	D 14±2	D 30±2	D 60±2	D 75±3
FCI Signature	X					
Complete medical history (general and ophthalmological)	X					

Somatometry (weight and height)	X	X	X	X	X	
Vital signs	X	X	X	X	X	
MC Evaluation	X	X	X	X	X	X
EA Assessment	X	X	X	X	X	X
Pregnancy test *	X				X	
ICO	X	X	X	X	X	
AVMC	X	X	X	X	X	
Assessment of ocular surface integrity (HC, Chemosis and TF)	X	X	X	X	X	
Goldmann ocular tonometry	X	X	X	X	X	
Gonioscopy	X	X				
Fundoscopy in mydriasis (evaluation of the optic nerve, its cup and the retina)	X	X			X	
Evaluation of eligibility criteria	X	X				
Photograph of the optic nerve	X	X			X	
Subject code assignment	X					
Delivery of the identification card	X					
Submission of the subject and training diary	X	X		X		
Delivery of the TC	X	X		X		
Return of the TC		X		X	X	
Assessment of treatment adherence (review of subject's diary and weighing of bottles)		X	X	X	X	
Corneal pachymetry		X			X	
Visual fields		X			X	
OCT of optic nerve and ganglion cells		X			X	
Randomization to experimental treatment group		X				
Delivery of experimental treatment		X		X		
Fundoscopy without mydriasis, unless the investigator considers mydriasis necessary (evaluation of the optic nerve, its cup and the retina)			X	X		
Return of experimental treatment				X	X	
Continuity assessment				X	X	

Abbreviations: ICF, Informed Consent Form; MC, concomitant medication; AE, adverse events; OCI, ocular comfort index; BCVA, best-corrected visual acuity; CH, conjunctival hyperemia; CT, concomitant treatment; TF, corneal fluorescein staining; OCT, optical coherence tomography; VE, screening visit; VB, baseline visit; V1-2, follow-up visit; VF, final visit; LlS , safety call. *When applicable.

3. 6 Randomization

At the baseline visit, after verification of the inclusion/exclusion criteria, eligible patients will be randomized according to a 1:1 ratio to KOF or EOF by means of random numbers generated by software (SAS Institute , Inc , Cary NC, USA), by an authorized third party.

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

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

Example:

A. Arieh Daniel Mercado Carrizalez
a. Initials: AMC

B. Juan De la Torre Orozco
b. Initials: JDO

3.7 Subject code assignment

Once the subject has been selected, they will be assigned a number that will identify them throughout the study. This code will consist of eight numbers in the following order from left to right:

- three digits of the molecule under study according to the name given by the sponsor.
- two digits corresponding to the research center number.
- three digits of the consecutive number assigned to its inclusion in the research center.

Example: 122-01-001

4. Sample Size Calculation

An estimated 58 evaluable cases (eyes) are estimated per treatment arm (participants may contribute one or two cases [eyes]), 116 cases in total.

The sample size calculation was based on the study by Joh and Jin (2019), where they evaluated the efficacy of a triple-MMT *versus* a double-MMT in reducing IOP after 1 year of follow-up [2].

This was a retrospective, consecutive case series study, in which 82 eyes of 82 subjects were included. Forty-five eyes received triple MMT consisting of tafluprost or brimonidine + brinzolamide/timolol (in a fixed combination) vs 37 eyes treated with tafluprost/timolol or brinzolamide/brimonidine (in a fixed combination). The primary efficacy variable was the decrease in mean IOP from baseline vs final. The reduction rate for the triple MMT group at 12 months was 52.7% vs 50.4% for the double MMT group. At three months, the IOP in triple MMT was 16.1 ± 2.2 mmHg vs 35.5 ± 4.5 mmHg at baseline, while for double MMT it was 16.5 ± 2.7 mmHg vs 33.7 ± 5.8 mmHg at baseline.

For the present protocol, a reduction in IOP after two months of treatment with 3 or 4 pharmaceutical agents of ≥ 20% with respect to their initial IOP with a single agent (latanoprost) is expected, with a difference between the groups of ≥ 1.5 mmHg. For the calculation, the difference of -2.0 ± 3.8 mmHg [2]at 3 months of treatment with respect to the initial value between triple-MMT and double-MMT was considered, expecting that in subjects treated with KOF + GOF the difference with respect to EOF + GOF would be 1.5 mmHg.

The relationship between the sample size of the two groups is:

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

The sample size was calculated using the equation for two means, to prove that the mean of group A (GOF + KOF) is greater than the mean of group B (GOF + EOF). [3]

The sample size and power were calculated using an online tool and following the equations described in proposal II. [4]The calculation was performed considering a power of 80% (β), a significance level of 0.05 (α), and a non-inferiority/superiority margin (δ) of 1.5.

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

$$1 - \beta = \Phi(z - z_{1-\alpha}) + \Phi(-z - z_{1-\alpha}) \; , z = \frac{\mu_A - \mu_B - \delta}{\sigma\sqrt{\frac{1}{n_A} + \frac{1}{n_B}}}$$

Where:

k= n_A / n_B is the coincidence relationship,

σ is the standard deviation

Φis the function of the standard normal distribution,

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

α is the Type I error,

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

δ is the test margin.

According to the previous calculation, the result was 45 cases (eyes); this estimate was increased by 30% to account for potential losses. The total suggested sample size is 58 cases (eyes) per treatment arm (116 eyes in total).

5. Clinical data management

Clinical data management (CDM) enables the generation of high-quality, reliable, and statistically valuable data. CDM is the process of collecting, cleaning, and managing subject information in a study in compliance with regulatory standards (*21* CFR Part 11, ICH, and GCP guidelines). It covers CRF design, CRF commenting, database design, *data entry , source* validation, and the creation of a database. *document verification* , SDV), discrepancy handling (*queries*), medical coding (*medical coding*), extraction (*soft lock*) and closing the database (*hard lock*) [5].

In accordance with roles and responsibilities, multiple users can be created, whose types of access to the CRF can be limited to data entry (principal investigator, PI), medical coding, database design , or *quality* control. *check*) [5, 6]. Discrepancy handling will be done based on the flow in Figure 2 :

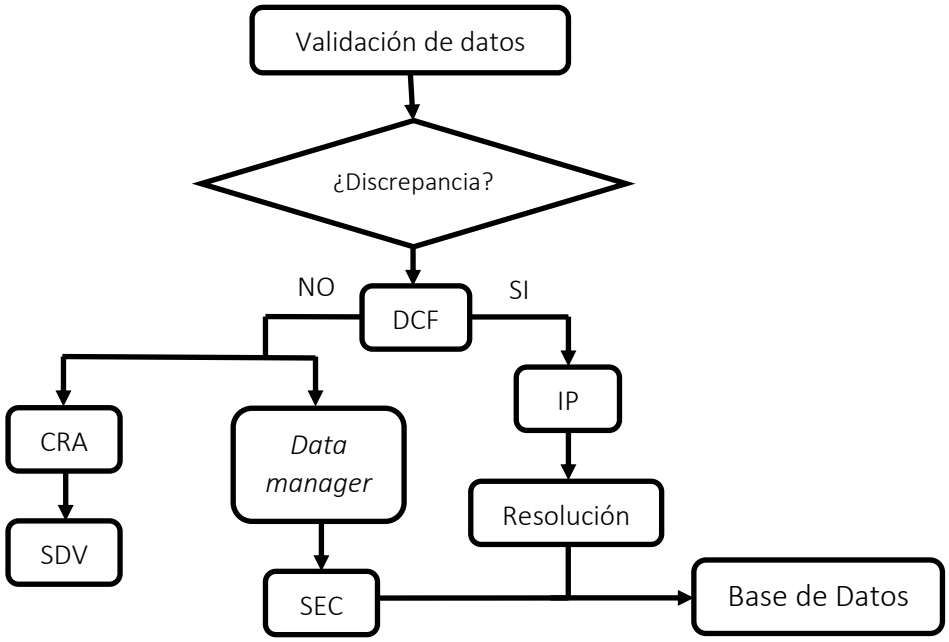

Figure 2. Discrepancy management . (DCF, medical note ; CRA, clinical monitor ; SEC, self-evident correction)

The CDM team will include the following roles:

- Data Manager,
- Database designer/programmer,
- Clinical coder (*Medical coder*),
- Clinical data coordinator,
- Quality control, and
- *Data* entry *associate*).

6. Statistical Methodology

The PAE was developed according to the evaluation criteria described in the study protocol. Statistical analysis will be performed by staff at Laboratorios Sophia, SA de CV. The statistical package SPSS version 19.0 for Windows (IBM Corporation, Armonk, NY, USA) will be used.

The personnel assigned to statistical data management will be blinded to the intervention groups. Coding will be performed using consecutive numbers for each intervention group. Data will be collected and organized in an Excel spreadsheet (Microsoft® Office). The data will then be exported to the SPSS software platform. Variables will be categorized according to their nature; see Table 2 .

Table 2. Operational Definition of the Variables

Variable	Conceptual Definition	Operational Definition	Type of measurement	Reference value	Statistical test
PIO	Tonometry is the objective measure of IOP, based primarily on the force required to flatten the cornea or the degree of corneal indentation produced by a fixed force.	Goldmann tonometry based on the Imbert-Fick principle [7]	Continuous quantitative	19 – 26 [1] mmHg / 9 – 26 [2] mmHg	Student's paired t-test Mann-Whitney U*
Proportion achieving IOP ≤13, ≤14, ≤15, and ≤18 mmHg	Frequency of subjects (percentage) who reach an IOP ≤13, ≤14, ≤15, and ≤18 mmHg at the end of the protocol, compared to their initial value	Classification : Yes/No	Nominal categorical	0 – 100	χ^2 or Fisher's exact test McNemar test*
Proportion that decreased IOP ≥ 20%, ≥ 25%, ≥ 30%, and ≥ 35%	Frequency of subjects (percentage) achieving an IOP reduction of ≥ 20%, ≥ 25%, ≥ 30%, and ≥ 35%, compared to their initial value	Classification : Yes/No	Nominal categorical	0 – 100	χ^2 or Fisher's exact test McNemar test*
Adverse events (AEs)	Any undesirable medical event that may occur in a research subject during the clinical research phase of a drug or vaccine but that does not necessarily have a causal relationship with it. [8]	The AEs expressed during the study will be collected through the CRF	Continuous quantitative Qualitative categorical	**Frequency:** Subjects presenting AE/Total number of exposed subjects **Intensity:** – Mild – Moderate – Severe **Causality:** – Unlikely related – Probably related – Possibly related – Related	Student's t test χ^2 or Fisher's exact test
Changes in the AVMC	MCVA is a test of visual function. Spatial VA is the ability to distinguish separate elements of an object and identify them as a whole.	Snellen chart. It is quantified as the minimum angle of separation (located at the nodal point of the eye) between two objects that allows them to be perceived as separate objects.	Discrete quantitative	Fraction: 20/20 – 20/200 Decimal: 0.1 – 1.0	Student's t test Mann-Whitney U*
Optic nerve size	Pathological excavation of the optic disc is generally associated with glaucoma	It will be performed by indirect ophthalmoscopy at a slit lamp; the lens will be chosen at the investigator's discretion. It can be performed under pharmacological mydriasis (tropicamide 0.8%/phenylephrine 5%).	Qualitative ordinal	– Little, – Medium, – Big, – Macrodisc	χ^2 or Fisher's exact test

Variable	Conceptual Definition	Operational Definition	Type of measurement	Reference value	Statistical test
Vertical cup-to-disc ratio (CDR)	The mean value of RCD is 0.25 – 0.3 and the difference between AO is less than 0.2	It will be performed by indirect ophthalmoscopy at a slit lamp; the lens will be chosen at the investigator's discretion. It can be performed under pharmacological mydriasis (tropicamide 0.8%/phenylephrine 5%).	Nominal categorical	0.1 to 0.95	Student's t test Mann-Whitney U*
Characteristics of the neuroretinal ring	It will be performed by indirect ophthalmoscopy at the slit lamp, the lens will be chosen at the discretion of the researcher.	Observation. May be performed under pharmacological mydriasis (tropicamide 0.8%/phenylephrine 5%).	Qualitative ordinal	– Concentric decrease – Sectoral or notched tapering – Mixed – Other	χ^2 or Fisher's exact test
Characteristics of the peripapillary area	It will be performed by indirect ophthalmoscopy at the slit lamp, the lens will be chosen at the discretion of the researcher.	Observation. May be performed under pharmacological mydriasis (tropicamide 0.8%/phenylephrine 5%).	Qualitative ordinal	– Sectoral atrophy-hypotrophy – Generalized atrophy-hypotrophy – Normal	χ^2 or Fisher's exact test
Evaluation of retina and macular area	Refers to the examination of the posterior segment. It will be graded as: normal and abnormal	Direct observation (biomicroscopy)	Nominal categorical	Normal	χ^2 or Fisher's exact test
Changes in corneal thickness	GCC measurement is an essential factor in determining the state of the cornea and the endothelial pumping mechanism. [9]	Central corneal pachymetry (specular microscope), under local topical anesthesia with tetracaine, the central corneal thickness will be measured.	Continuous quantitative	500 – 585 µm	Student's t test Mann-Whitney U*
Nerve fiber layer thickness (average)	Optical coherence tomography (OCT) will measure the thickness of the nerve fiber layer.	OCT, the baseline and final OCT will be performed with the same OCT, with the same protocol and the characteristics will be recorded along with the central average	Continuous quantitative	50 – 130	Student's t test Mann-Whitney U*
Nominal mean value (nerve fiber layer & ganglion cell thickness)	The average nominal value will be evaluated by means of OCT.		Qualitative ordinal	– Green, up to 95% CI – Yellow, up to 95% CI – Red, up to 95% CI	χ^2 or Fisher's exact test
Ganglion cell thickness			Continuous quantitative	≥ 45	Student's t test Mann-Whitney U*
Visual fields DM (mean deviation), dB	The visual field refers to the visual cortex's perception of objects	Computerized campimetry. Results included in the study will be considered reliable if	Continuous quantitative	-22 – 0	Student's t test

Variable	Conceptual Definition	Operational Definition	Type of measurement	Reference value	Statistical test
	and light sources at a particular moment with gaze fixation. The cortex's perception considers that the object or light source was processed by the visual pathway from a stimulus in the retina. [10]	they meet <20% fixation loss, false positives, and false negatives. [11]			Mann-Whitney U*
Severe central visual field loss (sensitivity ≤ 10 dB at ≥ 2 of the 4 points central to the fixation point of the visual field)			Nominal qualitative	No	χ^2 or Fisher's exact test
Shaffer gonoscopy (greater value of 2 or more sectors)	Using a Goldman gonoscope , the Shaffer classification will be performed to establish the open angle (grade III and IV) and its characteristics.	Direct observation with a Goldman gonocoposcope . The three-mirror Goldman lens will be placed on the cornea, after instilling topical tetracaine and 2% hypromellose as a lubricant. The quadrants will be observed and described, starting with the superior quadrant, then the temporal quadrant, the inferior quadrant, and ending with the nasal quadrant.	Qualitative ordinal	– 4 (45° – 35°) – 3 (35° – 20°) – 2 (20°) – 1 (≤ 10°) – 0 (0°)	χ^2 or Fisher's exact test
Corneal and conjunctival staining with fluorescein	Detection of epithelial defects in the conjunctiva and cornea	Direct slit-lamp observation, Oxford scale graduation. See Appendix 11. 1 Oxford scale The staining is presented in a series of panels (AE). The staining points range from 0-5 for each panel and from 0-15 for the total exposed area of conjunctiva and cornea.	Qualitative Ordinal	0 – V	χ^2 or Fisher's exact test McNemar test*
Conjunctival hyperemia changes	It is defined as the simplest reaction of the conjunctiva to a stimulus, a red appearance is observed secondary to the vasodilation of the vessels of the conjunctiva of variable intensity.	Direct observation. Classification using the Efron scale. See Appendix 11.2 Efron Scale for Conjunctival Hyperemia	Qualitative ordinal	Degrees: Normal – Severe	χ^2 or Fisher's exact test McNemar test*
Incidence of chemosis	It is defined as conjunctival edema resulting from an inflammatory reaction. It is classified as present or absent.	The evaluator will use a narrow beam of light at 60° and measure whether the conjunctiva separates by ≥1/3 of the entire eyelid opening or if it extends beyond the gray line.	Qualitative ordinal	Absent	χ^2 or Fisher's exact test McNemar test*
ICO	The ICO is a questionnaire designed to measure ocular surface irritation, assessing symptoms focused on	The evaluator will give the questionnaire to the subject and allow them to answer it calmly without any pressure or coercion. They will only assist them if they have	Discrete Quantitative	Score: 0 – 100	Student's t test Mann-Whitney U*

Variable	Conceptual Definition	Operational Definition	Type of measurement	Reference value	Statistical test
	discomfort associated with ocular surface disorders.	difficulty understanding any of the questions. See Appendix 11.3 Eye Comfort Index .			

Abbreviations: IOP, intraocular pressure; X^2, Chi-square; AE, adverse events; CRF, case report form; BCVA, best-corrected visual acuity; CDR, cup-to-disc ratio; CCT, central corneal thickness; OCT, optical coherence tomography; 95% CI, 95% confidence interval; OCI, ocular comfort index. *When applicable, [1] screening and baseline visit, [2] I – Final visits.

6.1 Population Analysis

Wilk (SW) tests will be performed as applicable (in case of n < 50), to determine whether the distribution presents normality in the results obtained in each study group [12].

The results of continuous quantitative variables will be presented as measures of central tendency: mean, standard deviation, and ranges. The analysis of nominal and ordinal qualitative variables will be presented as frequencies, proportions, and/or percentages.

The level of difference considered significant (p) will be an alpha (α) of 0.05 or less. A 95% confidence interval (95% CI) will be considered for the non-inferiority criteria [13]. The triangulation between the type of variables and the measurements is shown in Table 3 .

The efficacy analysis will be performed on patients randomly assigned to an intervention group who did not experience protocol deviations at their final visit (per-protocol population, PP). The safety and tolerability analysis will consider all subjects randomly assigned to an intervention group (intention-to-treat population, ITT).

6.2 Effectiveness Analysis

The statistical analysis of continuous quantitative variables to find significant differences will be as follows:

- Intra-group analysis : Student's t-test, or Mann-Whitney U for KS <0.05.
- Between-group analysis : differences between groups will be analyzed using the Student t-test for independent groups or the Mann-Whitney U test when appropriate (KS <0.05).

Those subjects who complete all their visits and have adherence to treatment by weight and diary ≥ 90% will be included in the statistical analysis to meet the objective of the study (protocol population, PP).

Statistical analysis to identify significant differences in qualitative variables will be performed by creating 2x2 contingency tables as follows:

- Intra-group differences : These will be determined using the McNemar test [14]. This test is applied to 2x2 contingency tables with a dichotomous trait, with matched subject pairs, to determine whether the marginal frequencies of the row and column are equal (marginal homogeneity).
- Difference between groups : differences between groups will be analyzed using Pearson's Chi-square (X^2) test or Fisher's exact test for expected values less than 5.

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

The investigational product will be considered effective when there are no clinical and statistical differences in the primary outcome variable, with respect to its comparator (GOF + EOF), considering a 95% confidence interval (95% CI) for the non-inferiority criteria based on the study objectives .

Table 3. Triangulation of concepts

Variable type	Variable	A1	A2	A3	A4	B1	B2	D1	D2	D3	D4	D5	D6	D7	E1
Scrutiny															
A1	Demographics	DT													
A2	CT evaluation		DB											TB	
A3	Medical History & Inclusion Criteria			D	D	B									
A4	Comprehensive Ophthalmological Evaluation (RCD, SP & SA)			D	TB				TB					TB	
Effectiveness															
B1	Ocular Tonometry			D	D	BM	M							TB	
B2	Changes in Ocular Tonometry, %					TM	TM							TB	
Security															
D1	AVMC			D				BM						TB	
D2	RCD, SP & SA			D	TB				TM					TB	
D3	GCC			D						B				TB	
D4	Campimetry				B							M		TB	
D5	Gonioscopy				D							TB		TB	
D6	Ocular surface integrity (HC, Chemosis, TF)				D							TM	TB	TB	
D7	EA		T			T		T	T	T	T	T	T	TB	
Tolerability															
E1	ICO														M

Abbreviations: CT, concomitant treatment; CDR, cup-to-disc ratio; PS, posterior segment; SA, anterior segment; BCVA, best-corrected visual acuity; CCT, central corneal thickness; HC, conjunctival hyperemia; TF, fluorescein staining; AE, adverse events; OCI, ocular comfort index; D, descriptive statistics; T, 2x2 contingency table; B, bivariate analysis ; M, multivariate analysis.

6.3 Safety & Tolerability Analysis

The safety and tolerability assessment will include in the analysis all subjects who have been exposed to any of the interventions, regardless of the visit at which they were eliminated from the study (intention-to-treat (ITT) population).

For the analysis of safety and tolerability variables, the same primary analysis will be performed as long as the variables are continuous and have the necessary measurements. The differences between qualitative variables will be determined by creating 2x2 contingency tables, using Pearson's $X^{2\ statistic}$ or Fisher's Exact statistic, for expected frequencies less than 5.

For the reporting of adverse events, those participants who were randomly assigned to an intervention group after application of the investigational product (ITT population) will be considered.

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

6.4 Other analyses

According to the sample size calculation to meet the study objective, 116 evaluable cases (58 cases per arm) are required. If this number is not met due to a loss of subjects exceeding the 30% threshold considered in the sample size calculation for this protocol (loss to follow-up or withdrawal from ICF), the sponsor may substitute these subjects to balance the treatment groups.

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

In case it is required to test the conditional association of two binary variables in the presence of a third categorical variable, the Cochran-Mantel-Haenszel (CMH) test will be used.

6.5 Analysis by treatment arm

For quantitative variables:

- Intra-group analysis : Student's t-statistic for repeated measures, or sign test in case of SW $p<0.05$.
- Between-group analysis : Student's t statistic or Mann-Whitney U in case of SW $p<0.05$.

For qualitative variables:

- Intra-group difference : McNemar's test. This test is applied to 2x2 contingency tables with a dichotomous trait, with matched pairs of subjects, to determine whether the marginal frequencies of the row and column are equal (marginal homogeneity) [14].
- Difference between groups : Pearson's X2 test ^{or} Fisher's exact test for expected values less than 5.

7. Change Control

1. The content of the cover page was modified and the version of the protocol was updated.
2. The objectives and hypotheses of the study were modified.
3. Table 1 : Study schedule was modified.
4. Inclusion and Exclusion criteria were modified.
5. The Efficacy and Safety variables were modified.
6. The Clinical Data Management section was included.
7. Table 2: Operational definition of variables was modified.
8. Table 3: Triangulation of concepts was modified.
9. Exploratory analyses were eliminated and other analyses and analyses by treatment arm were included.
10. The general structure of the document was modified.

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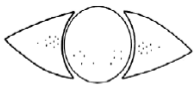
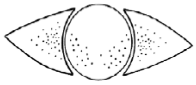
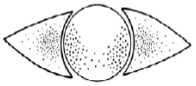
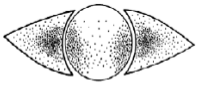
9. Annexes

9.1 Oxford Scale

PANEL	Grade	Criteria
A 	0	Igual o menor que el panel A
B 	I	Igual o menor que el panel B, mayor que el A
C 	II	Igual o menor que el panel C, mayor que el B
D 	III	Igual o menor que el panel D, mayor que el C
E 	IV	Igual o menor que el panel E, mayor que el D
>E	V	Mayor que el panel E

9.2 Efron Scale

9.3 Eye Comfort Index

Índice de Confort Ocular

Ficha de identificación

No. de estudio: SOPH122-0420/IV _____ **Fecha:** ___/___/___

Iniciales del sujeto: _____ **No. de sujeto:** 122-_____-_____

Indicaciones:

Este cuestionario fue diseñado para calificar el confort de sus ojos.
Para cada pregunta circule su respuesta

Ejemplo: En la semana pasada, ¿qué tan seguido sus ojos estuvieron rojos?

Nunca						Siempre
0	1	2	3	4	5	6

No existen respuestas correctas o incorrectas. No tome demasiado tiempo en cada pregunta.

1 En la semana pasada, ¿qué tan seguido sus ojos se sintieron *secos* ?

Nunca						Siempre
0	1	2	3	4	5	6

Cuando sus ojos se sentían *secos* , por lo general, ¿qué tan intensa era la sensación?

No lo he sentido						Severo
0	1	2	3	4	5	6

2 En la semana pasada, ¿qué tan seguido sus ojos se sintieron *arenosos* ?

Nunca						Siempre
0	1	2	3	4	5	6

Cuando sus ojos se sentían *arenosos* , por lo general, ¿qué tan intensa era la sensación?

No lo he sentido						Severo
0	1	2	3	4	5	6

3 En la semana pasada, ¿qué tan seguido sus ojos sintieron *punzadas* ?

Nunca						Siempre
0	1	2	3	4	5	6

Cuando sus ojos sentían *punzadas* , por lo general, ¿qué tan intensa era la sensación?

No lo he sentido						Severo
0	1	2	3	4	5	6

4 En la semana pasada, ¿qué tan seguido sus ojos se sintieron *cansados* ?

Nunca						Siempre
0	1	2	3	4	5	6

Cuando sus ojos se sentían *cansados* , por lo general, ¿qué tan intensa era la sensación?

No lo he sentido						Severo
0	1	2	3	4	5	6

Hoja 1 de 2

Índice de confort ocular

5 En la semana pasada, ¿qué tan seguido sus ojos se sintieron *adoloridos* ?

Nunca						**Siempre**
0	1	2	3	4	5	6

Cuando sus ojos se sentían *adoloridos* , por lo general, ¿qué tan intensa era la sensación?

No lo he sentido						**Severo**
0	1	2	3	4	5	6

6 En la semana pasada, ¿qué tan seguido sus ojos sintieron *comezón* ?

Nunca						**Siempre**
0	1	2	3	4	5	6

Cuando sus ojos sentían *comezón* , por lo general, ¿qué tan intensa era la sensación?

No lo he sentido						**Severo**
0	1	2	3	4	5	6