Creation date: march 2016

A phase III clinical study, to evaluate the non-inferiority in the intraocular pressure decrease of the preservative-free ophthalmic solution PRO-122, manufactured by Laboratorios Sophia S.A. de C.V., versus concomitant therapy in subjects with uncontrolled primary open-angle glaucoma and/or intraocular hypertension

Protocol code: SOPH122-0316/III

Protocol version: 2.0

Version date: 25/01/2017

Register: Pending

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

1. Overview

Title of the study: A phase III clinical study, to evaluate the non-inferiority in the intraocular pressure decrease of the preservative-free ophthalmic solution PRO-122, manufactured by Laboratorios Sophia S.A. de C.V., versus concomitant therapy in subjects with uncontrolled primary open-angle glaucoma and/or intraocular hypertension	
Protocol code: SOPH122-0316/III	**Creation date:** 03/30/2016
Protocol version: 2.0	**Version date:** 25/01/2017
Therapeutic indication: Ocular hypotensive	
Study period: 9 months	**Clinical study phase:** III
Objectives: To evaluate the non-inferiority in the intraocular pressure decrease of the preservative-free ophthalmic solution PRO-122, manufactured by Laboratorios Sophia S.A. de C.V., versus concomitant therapy in subjects with uncontrolled POAG and/or IOP.	
Hypothesis: The mean (average) value of the IOP final absolute reduction in the experimental group (PRO-122) is not lower, considering a lower limit of 1 mmHg, compared to the IOP mean absolute reduction of the standard group (concomitant therapy).	
Methodology: A non-inferiority, phase III, double-blind, randomized, controlled, parallel, clinical trial	
Number of patients: 51 subjects divided into 3 groups (17 subjects per group)	
Diagnosis and main inclusion criterion: Diagnosis: Primary open-angle glaucoma or ocular hypertension Main criteria: - Patients of either sex - Average intraocular pressure (IOP) ≤ 36 mm/Hg - Previous management with ocular hypotensive medications ≥ 2 months, without achieving control (target IOP) - Age ≥ 18 years - Informed consent	
Test product, dosage and route of administration: - PRO-122. Preservative-free ophthalmic solution of timolol 0.5% / brimonidine 0.2% / dorzolamide 2% Manufactured by Laboratorios Sophia, S.A. de C.V., Zapopan, Jalisco, Mexico. + Placebo + Placebo - Dosage: 1 drop every 12 hours - Route of administration: ophthalmic	
Treatment duration: 90 days	

Reference product, dosage and route of administration:

1. Krytantek Ofteno®. Timolol 0.5% / brimonidine 0.2% / dorzolamide 2% ophthalmic solution. Manufactured by Laboratorios Sophia, S.A. de C.V., Zapopan, Jalisco, Mexico. + Placebo + Placebo
    a. Dosage: 1 drop every 12 hours
    b. Route of administration: ophthalmic
2. Concomitant triple therapy. Imot, timolol 0.5% ophthalmic solution, manufactured by Laboratorios Sophia, S.A. de C.V. + Alphagan, brimonidine 0.2% ophthalmic solution, manufactured by Allergan, Inc. + Trusopt dorzolamide 2% manufactured by Merck Sharp & Dohme Corp.
    a. Dosage: 1 drop every 12 hours
    b. Route of administration: ophthalmic

Evaluation criteria:

Efficiency (non-inferiority):

- IOP decrease

Safety:

- Best corrected visual acuity
- Cup-to-disc ratio
- Visual fields determined by computerized perimetry
- Central corneal thickness determined by pachymetry
- Ocular surface integrity, including:
    - Conjunctival hyperemia
    - Chemosis
    - Fluorescein staining
- Density of goblet cells
- Adverse events

Tolerability:

- Ocular comfort index

Statistical methodology:

The data will be expressed with measures of central tendency: mean and standard deviation for quantitative variables. The qualitative variables will be presented in frequencies and percentages. Statistical analysis will be done by means of a Kruskal-Wallis test for quantitative variables. The difference between qualitative variables will be analyzed using an $\chi 2$ (Chi2). An alpha ≤ 0.05 would be considered as significant.

Contenido

1. OVERVIEW 2

2. CONTENTS 4

3. LIST OF ABBREVIATIONS 8

4. ADMINISTRATIVE STRUCTURE OF THE STUDY 9

5. INTRODUCTION 12

5.1 THEORETICAL FRAMEWORK 12
5.2 PROBLEM DEFINITION AND RATIONALE 13
5.3 BACKGROUND 13
5.3.1 EFFICACY 13
5.3.2 SAFETY 14
5.3.3 PRESERVATIVE USE IN OCULAR HYPOTENSIVE MEDICATIONS 20
5.4 JUSTIFICATION 21
5.5 OBJECTIVES AND HYPOTHESIS 21
5.5.1 GENERAL OBJECTIVE 21
5.5.2. SPECIFIC OBJECTIVES 21
5.5.3 HYPOTHESIS 21
5.6 STUDY PLAN AND DESIGN 22
5.6.1 DISCUSSION OF THE STUDY DESIGN 22

6. MATERIAL AND METHODS. PARTICIPANTS, INTERVENTIONS AND VARIABLES 23

6.1 STUDY CENTER 23
6.1.2 CENTER ORGANIZATION 23
6.1.3 DOCUMENTATION TO BE DELIVERED TO THE SPONSOR 24
6.1.3 CENTER CLOSURE 24
6.2 ELEGIBILITY CRITERIA 24
6.2.1 INCLUSION CRITERIA 24
6.2.2 EXCLUSION CRITERIA 24
6.2.3 ELIMINATION CRITERIA 25
6.2.4 SUBJECT IDENTIFICATION 26
6.3 INTERVENTION 26
6.3.1 GIVEN TREATMENTS 26
6.3.2 STRATEGIES TO IMPROVE THE ADHERENCE AND PROCEDURE FOR MONITORING THE ADHERENCE 28
6.3.3 ALLOWED AND PROHIBITED CONCOMITANT TREATMENTS AND INTERVENTIONS BEFORE AND AFTER THE STUDY 29

6.3.4 TREATMENT MANAGEMENT 30
6.4 OUTCOME VARIABLES 31
6.4.1 SAFETY VARIABLES 31
6.4.2 EFFICACY VARIABLES 32
6.4.3 METHODS AND GRADINGS TO BE USED IN THE MEASUREMENT OF VARIABLES 32
6.4.4 MEASUREMENTS BY STAGE 35
6.5 CHRONOGRAM AND STUDY DIAGRAM 37
6.5.2 PROCEDURES TO FOLLOW IN EACH VISIT 38
6.5.3 STUDY DIAGRAM 41
6.6 SAMPLE SIZE 41
6.6.1 SAMPLE SIZE CALCULATION 41
6.7 RECRUITMENT 42

7. METHODS. INTERVENTION ASSIGNMENT 42

7.1 ASSIGNMENT SEQUENCE GENERATION 42
7.2 BLINDING MECHANISM 42
7.3 IMPLEMENTATION 43
7.4 BLINDING (MASKING) 43
7.4.1 BLINDING OPENING 43

8. METHODS. COLLECTION, ADMINISTRATION AND DATA ANALYSIS. 43

8.1 DATA RECOLLECTION METHODS 43
8.1.1 FOLLOW-UP COMPLETION STRATEGIES 44
8.2 DATA ADMINISTRATION 44
8.3 STATISTICAL METHODOLOGY 44
8.3.1 PRIMARY AND SECONDARY OUTCOME VARIABLES 44
8.3.2 ADDITIONAL ANALYSIS 45
8.3.3 POPULATION ANALYSIS AND MISSING DATA MANAGEMENT 45

9. METHODS. MONITORING 46

9.1 MONITOREO DE DATOS 46
9.2 PRELIMINARY ANALYSIS AND EARLY STUDY TERMINATION 47
9.3 ADVERSE EVENTS 47
9.3.1 INVESTIGATOR RESPONSIBILITIES 47
9.3.2 RESPONSABILITIES OF THE SPONSOR 52
9.4 AUDIT 52
9.4.1 STUDY PRE-AUDIT 52
9.4.2 AUDIT/INSPECTION DURING THE CONDUCT OF THE TRIAL 52

10. ETHICAL CONSIDERATIONS 52

10.1 APPROVAL OF THE COMMITEES 52

10.2 AMENDMENTS TO PROTOCOL 53
10.3 CONSENT 54
10.3.1 OBTAINING 54
10.3.2 SPECIAL CONSIDERATIONS 54
10.3.3 MODIFICATION TO THE INFORMED CONSENT 54
10.4 CONFIDENTIALITY 55
10.5 DECLARATION OF INTERESTS 55
10.6 ACCESS TO INFORMATION 55
10.7 AUXILIARY AND POST-STUDY-TERMINATION CARES 55
10.8 BIOSAFETY ASPECTS 55
10.9 FINAL REPORT AND RESULT PUBLICATION 56
10.9.1. FINAL REPORT 56
10.9.2 DISCLOSURE OF RESULTS 56
10.9.3 PUBLICATION OF RESULTS 56

11. REFERENCES 57

12. SIGNATURE PAGE 64

13. ANNEXES 65

13.1 OCULAR COMFORT INDEX 66
13.2 EFRON SCALE FOR CONJUNCTIVAL HYPEREMIA 68
13.3 OXFORD SCALE 68

List of tables and figures

Table 1. Administrative structure 10
Table 2. Adverse events in the study by Konstas 16
Table 3. Adverse events in the study by Realini 18
Table 4. Adverse events in the study by Baiza 2009 18
Table 5. Adverse events in the study by Baiza 2012 19
Table 6. Adverse events in the study by García 20
Table 7. PRO-122 qualitative and quantitative formulation 27
Table 8. Qualitative/quantitative formulation of Krytantek Ofteno® 28
Table 9. Washout Period 30
Table 10. Method for the measurement of variables 32
Table 11. Study Chronogram 38
Table 12. Karch and Lasagna's algorithm modified by Naranjo 51

Figure 1. Study design 22
Figure 2. Minimal organization of the Center 23
Figure 3. Impression cytology 34
Figure 4. Study diagram 41
Figure 5. Attention to the adverse event 49

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3. List of abbreviations

AA	Alpha-adrenergic agonist
AE / SAE	Adverse event / Serious adverse event
BAK	Benzalkonium chloride
BB	Beta-blocker
BCVA	Best corrected visual acuity
BID	Twice a day
CAIs	Carbonic anhydrase inhibitors
CCT	Central corneal thickness
CRF	Case Report Form
FDA	Food and Drug Administration
GCP	Good clinical practice
IC	Informed consent
ICH	International Conference on Harmonization
ICL	Informed consent letter
IOH	Intraocular hypertension
IOP	Intraocular pressure
MAO	Monoamine oxidase
MD	Mean deviation
MTMT	Maximal tolerated medical therapy
OCI	Ocular comfort index
PGA	Prostaglandin analogue
PI	Principal investigator of the clinical study
POAG	Primary open-angle glaucoma
QD	Once a day
REC	Research Ethics Committee
SDM	Standard deviation of the model
SDV	Source document verification
TID	Three times a day

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4. Administrative structure of the study

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

Function	Name / contact	Affiliation¥
Doctor in charge of the study	Dr. Leopoldo Martín Baiza Durán leopoldo.baiza@sophia.com.mx	Regulatory Affairs and Medical Director
Study Director	Dr. Aldo Arturo Oregón Miranda aldo.oregon@sophia.com.mx	Clinical Operations Manager
Scientific Committee	Dr. Oscar Olvera Montaño oscar.olvera@sophia.com.mx	Ophthalmologist investigator
Scientific Committee	Sc.D. Arieh Roldán Mercado Sesma arieh.mercado@sophia.com.mx	Medical writer
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International monitor	M. en F. Sandra Carolina Gómez Méndez sandra.gomez@sophia.com.mx	Senior clinical research associate
Monitor	QFB Jessica Lizette Mejía Gutiérrez jessica.mejia@sophia.com.mx	Senior clinical research associate
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Monitor	QFB Lizeth de Jesús Pérez Lerma lizeth.perez@sophia.com.mx	Junior clinical research associate
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Table 1. Administrative structure

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5. Introduction

5.1 Theoretical framework

Glaucoma is a progressive optic neuropathy characterized by the loss of retinal ganglion cells and its axons, which results in a distinctive optic disc appearance and a concomitant loss of the visual function. [1] It is the second cause of blindness worldwide and, it is estimated that by 2020, 79.6 millions of people will have glaucoma, of which 74% will have primary open-angle glaucoma (POAG). [2] The Latin American population, especially those of Mexican origin, are more likely to develop POAG compared to white population. [3] [4]

The mechanism by which the glaucoma damages the optic nerve is probably multifactorial; however, high intraocular pressure (IOP) is the main risk factor and the only one that can currently be modified to prevent the damage progression by glaucoma, including normotensive glaucoma [5] [6] [7] [8] [9]. It has also be shown that IOP reduction decreases the conversion index of ocular hypertension (OH) to glaucoma.[7] Chauhan *et al.* have reported that for each 1 mmHg increase in the IOP, the risk of glaucoma progression increases in a 19%. [5]

Despite the great advances in the filtering surgical treatments, implants and laser procedures that improve the trabecular drainage, the pharmacological therapy remains as the initial intervention for most of patients with OH and glaucoma; this treatment typically includes the topical application of antihypertensive agents. [10]

At present, pharmacological options for the IOP reduction with topical application include prostaglandin analogues (PA), beta-blockers (BB), alpha-adrenergic agonist (AA), carbonic anhydrase inhibitors (CAIs) and parasympathomimetic drugs. Commonly, pharmacotherapy starts with a single-agent hypotensive application, generally of those referred as first-line drugs (prostaglandin analogues or beta-blockers). [11] [8]However, monotherapy can be insufficient in many cases, due to its inability to reach the target IOP and/or prevent glaucoma progression. In some other cases, a single drug can lose its effectiveness through time, due to Tachyphylaxis. The 5-year Ocular Hypertensive Treatment Study reported that about 40% of patients require two drugs to achieve an IOP reduction of 20% compared to baseline IOP, while an additional 9% needs more than two drugs. [7] [5] Thus, more than one drug is often required to control properly the IOP in the medium and long term. This can be achieved by the concurrent use of two or more drugs of different classes, either through its concomitant application or in a fixed combination. [12]

In addition to the efficiency to reduce the IOP, fixed combination offer multiple benefits when compared to the concomitant application of its active principles: [13] 1) a lower cost, 2) a simpler treatment regimen, 3) better adherence to treatment, and 4) decreasing of washout risk. [14] [15]

Nevertheless, few studies have compared directly the adherence to fixed combinations versus the concomitant use of its active principles, but there is evidence that adherence to glaucoma treatment is better when the regimen is simpler.[16] [17]

The theoretical support of drugs washout is based on the physiological function of the lacrimal apparatus. The tear film has an approximate volume of 8 to 10 µl and the lacrimal flow index is of 0.5 to 2.2 µl/min, which means that, in general, a replacement of the total volume of the tear film can be made every 10 minutes in average. Eye drops volume is between 25 and 50 µl, a volume that exceeds the capacity of contention that the ocular surface has, so that the excess is quickly drained by nasolacrimal duct or spilled at flickering. [18]

Fixed combinations also offer a risk decrease of corneal damage and ocular surface, related to the cumulative exposure of preservatives. For example, chronic exposure to benzalkonium chloride

(BAK), used in a large quantity of ophthalmic drugs, has been associated with inflammation, corneal and conjunctival damage, tear film disorders and symptomatology of ocular surface disease. [19] [20] [21]

Currently, available fixed combinations include a timolol BB 0.5% combined with a drug from other therapeutic families, a PGA, an AA or a topical CAI [22] In Mexico and other South American countries, a triple fixed-dose combination therapy also exists, which includes timolol, brimonidine and dorzolamide.

5.2 Problem definition and rationale

For the OH and POAG treatment, the use of a single drug may result insufficient when trying to achieve the target IOP, and must therefore rely on a use of 2 or more ocular hypotensive of different families.

In a simplified scheme for glaucoma treatment, it is proposed to start with monotherapy, based on a first-line drug like a PGA or BB, where the PGA is predominantly chosen by the ophthalmologists, nowadays. If this first therapy is not sufficient but responsive, a second drug is added, preferably another not-previously used first-line drug. In this intermediate therapy, the patient would be already receiving 3 to 4 instillations daily by, in theory, engaging its adherence to the treatment and exposing the patient to a higher cumulative dose of BAK. So that the attending physician may decide to change it to a fixed combination of two drugs. If the target IOP is not achieved, a third drug can be added to reach the maximal tolerated medical therapy (MTMT) stipulated by *Zimmerman* of 3 drugs in 3 daily instillations. [23]

As it has been already established, the use of fixed combinations offers benefits over the concomitant application of its components. To our knowledge, there are no reports about the evaluation of a triple fixed-dose combination therapy versus the concomitant application of its components. The triple fixed-dose combination therapy can increase treatment adherence with MTMT and extend the horizon toward the new MTMT plan proposed by *Sampaolesi*, which includes the triple fixed-dose combination therapy + a PGA. [23]

5.3 Background

5.3.1 Efficacy

The effectiveness of fixed combinations has been evaluated to obtain their regulatory approval. To get the US Food and Drug Administration (FDA) approval of a fixed combination, this must have a greater efficiency than the one of each of its components used as monotherapy. In addition, it must be as effective as its components, concomitantly administered. [14]

5.3.1.1 Timolol-Dorzolamide fixed combination

Two independent, randomized and double blind studies compared the fixed combination of timolol 0.5% / dorzolamide 2% versus a monotherapy with it components. *Boyle*'s study was conducted with 355 patients prior to drug washout. The study by *Cineschmidt* was carried out with 253 patients after the 3-week *run-in* of timolol. Posology for the fixed combination and timolol was BID and TID, for dorzolamide. The treatment time was of 3 months. Both authors concluded that the fixed combination was more effective in controlling IOP that monotherapy with any of the components. [24] [25]

Other studies have compared the fixed combination versus concomitant application of timolol-dorzolamide. *Hutzelmann* conducted a randomized, double blind, 3-month study with 299 patients and a run in period of 2 weeks with timolol. The posology was BID for both arms. Based on the

averages of months 2 and 3, the fixed and concomitant combinations were equivalent regarding the hypotensive effect, with an intergroup difference of IOP average of <0.1 mmHg. [26]

In contrast, *Strohmaier* reported in his study (n = 242 patients) that the fixed combination was approximately 1 mmHg less effective than the concomitant combination. [27]

5.3.1.2 Timolol-Brimonidine fixed combination

In a double-blind, randomized, 12-month study with 1159 patients with glaucoma or OH, the fixed combination of timolol 0.5% / brimonidine 0.2% was compared against the monotherapy of its components. Throughout the study, the fixed combination was better than monotherapy. [28]

Goni reported that fixed combination was as effective as the concomitant therapy with an intergroup difference of ≤ 0.30 mmHg. The study was conducted with 371 patients for 12 weeks. [14] [29]

5.3.1.3 Timolol-Brimonidine-Dorzolamide fixed combination

The triple fixed-dose combination has been evaluated in several studies and has been compared versus double fixed-dose combinations. A randomized, double-blind study with 112 patients compared the BID application for 180 days of triple fixed-dose combination therapy versus fixed timolol 0.5% / dorzolamide 2% combination. The triple combination was superior with a 3.4 mmHg difference in the IOP average of month 3. [30]

Another study compared the triple fixed-dose combination versus the fixed combination of timolol 0.5% / brimonidine 0.2%. It was a double blind, randomized study in patients with glaucoma and/or ocular hypertension, with a BID application for 3 months. The triple combination was better with a 2.16 mmHg difference in the IOP average of month 3 at the 8:00-hour revision. [31]

Currently, there is no information about the comparison of the triple fixed-dose combination with the concomitant application of its three components.

5.3.2 Safety

5.3.2.1 Phase IV clinical studies reports

In the study of *Babic N et al.*, the safety profile of fixed combinations fixed of travoprost / timolol and dorzolamide / timolol was assessed in 60 subjects (divided into 2 intervention groups, n = 30 subjects per group) with POAG or OH, who received 1 drop of QD and 1 drop of BID, respectively, for 3 months. Results: Four subjects were removed from the dorzolamide / timolol group due to a lack of complete data. No serious adverse events were recorded during the intervention period with the study drugs. Non-serious adverse events reported in the group exposed to travaprost / timolol (n = 30) were: hyperemia, blurred vision and pruritus. Hyperemia occurred in 50% of the participants, and blurred vision and pruritus occurred in a 6.7%. No subject of study reported eye dry or foreign body sensation. No abnormalities were either notified regarding taste perception.

In the group exposed to dorzolamide/timolol (n = 26), the most frequent adverse event was dry eye sensation, which affected 30.8% of the participants and the sensation of foreign body affected 23.1%. No subject reported hyperemia. A 3.8% of the patients presented dysgeusia.

In addition, no patient presented changes in iris pigmentation, changes in eyelashes, cystoid macular edema nor systemic disorders, which may affect the cardiovascular or respiratory system. Furthermore, nobody suspended the study drug, nor were they affected in their daily activities due to an adverse event. Therefore, both drugs were safe and well tolerated by the study patient population. [32]

In another 2-period, 2-sequence, crossover clinical trial conducted by the research team of *Konstas A. et al.*, the safety and tolerability profile of 2 ophthalmic formulations in a fixed combination of dorzolamide/timolol and brimonidine/timolol were evaluated. These formulations were given to patients with mild to moderate primary open-angle glaucoma or recently diagnosed ocular hypertension, who have not previously received any therapy, for 60 days. In this study, 77 patients were selected in the screening stage. Seven of these were excluded (4 decided not to participate and 3 did not complete the inclusion criteria). Seventy subjects started the adherence-assessment period by the administration of timolol0.5%, 5 from which were withdrawn (3 due to intraocular hypertension < 18 mmHg, and 2 due to an IOP reduction of < 20%). Sixty five subjects in total were randomized into 2 groups to receive fixed therapies. Period 1: in the dorzolamide / timolol group, 32 patients entered and in the brimonidine / timolol group, 33 patients entered. Five subjects were withdrawn due to adverse events: Two subjects did not tolerate the investigational medicinal product of dorzolamide/timolol and, for the group that received brimonidine/timolol, 1 patient presented hypotension and 2 patients tolerated the investigational medicinal product. In the second period, 30 subjects completed the evaluation in each intervention group.

Results: No drug-related serious adverse events were investigated during the entire period of study (see **Table 2 Adverse events in the study by Konstas**). In the group that received dorzolamide/timolol, 18.3% of the patients presented bitter taste, 16.7% of patients presented fluorescein staining in the ocular surface and 5% referred a conjunctival hyperemia.

From the group of patients receiving brimonidine/timolol, 16.7% had conjunctival hyperemia.

In the general analysis, both study drugs were well tolerated by the participating patients. The ocular adverse events reported were of a mild intensity and similar in both study groups, except for hyperemia which was higher in the group with brimonidine/timolol, and regarding staining and changes in taste that were higher in the individuals exposed to dorzolamide / timolol. [33]

In the analysis of 2 phase III clinical trials developed by *Realini T et al.*, the safety profile of the fixed combined formulation of brinzolamide 1% / brimonidine 0.2% versus monotherapies with brinzolamide 1% and brimonidine 0.2% was evaluated in subjects with primary open-angle glaucoma or ocular hypertension. Such therapies were administered in the affected eye TID for 3 months.

Results: In the study, 1350 patients were enrolled, who were randomize in 3 study groups: the group exposed to brimonidine had 452 patients, the group with brinzolamide had 458 patients and the group with a fixed-dose combined therapy had 433 patients. In the brimonidine group, 60 patients were withdrawn from the study: 35 due to adverse events, 8 due to voluntary withdrawal, 1 due to lack of follow-up, 1 due to an investigation protocol violation and 15 subjects due to an inappropriate control of IOP. In the brinzolamide group, 15 subjects were withdrawn from the analysis due to an adverse event, 3 due to a voluntary withdrawal, 3 due to an investigation protocol violation and 8 due to an inappropriate control of IOP. From the group with a fixed-dose combined therapy, 40 patients were withdrawn due to an adverse event, 4 due to a voluntary withdrawal, 1 due to lack of follow-up, 2 due to non-compliance to the posology, 3 due to an investigation protocol violation and 2 due to an inappropriate control of intraocular pressure.

Adverse event	Dorzolamide/timolol N (%)	Brimonidine/timolol N (%)	P
Non-ocular			
Bitter taste	11 (18.3)	0 (0)	0.001
Systemic hypotension	0 (0)	4 (6.7)	0.125
Dry mouth	0 (0)	4 (6.7)	0.125
Fatigue	1 (1.7)	3 (5.0)	0.625
Headache	0 (0)	1 (1.7)	1.000
Dizziness	0 (0)	1 (1.7)	1.000
Somnolence	0 (0)	1 (1.7)	1.000
Ocular			
Stainings	10 (16.7)	1 (1.7)	0.012
Conjunctival hyperemia	3 (5.0)	10 (16.7)	0.039
Pruritus	1 (1.7)	7 (11.7)	0.070
Lacrimation	1 (1.7)	2 (3.3)	1.000
Keratitis punctata	1 (1.7)	0 (0)	1.000
Foreign body sensation	0(0)	3 (5.0)	0.250
Dry eye sensation	1 (1.7)	3 (5.0)	0.625
Secretion	0 (0)	1 (1.7)	1.000
Palpebral edema	1 (1.7)	0 (0)	1.000
Burning	4 (6.7)	0 (0)	0.125

Adverse events reported with the use of fixed-dose combination therapies in participating patients. Note: Several patients experienced multiple adverse events. Abbreviations: p = statistical value

Table 2. Adverse events in the study by Konstas

A total of 272 patients experienced at least one adverse event (mostly ocular) associated to the investigational product (see table **Table 3. Adverse events in the study by Realini**): 107 (24.6%) subjects in the brimonidine/brinzolamide group, 86 (18.7%) of the brinzolamide group and 79 (17.4%) patients in the brimonidine group. Twenty patients presented 29 serious adverse events, 1 of which was related to the investigational drug, according to the investigator judgement; it was precordial pain of moderate intensity in a subject from the brinzolamide group, which resulted in the withdrawal of the patient and the clinical follow-up after the clinical picture remission.

Patients exposed to brinzolamide had a higher incidence of blurry vision (5.3 to 6.5%), and dysgeusia (3.9 to 8.3%) compared with the brimonidine group (0.2% in both studies). Patients receiving brimonidine showed higher incidence of ocular hyperemia (2.1 to 3.3%), dry mouth (2.4 to 3.0%), and ocular allergy (1.1 to 2.5%) compared to the group exposed to brinzolamide (0.7, 0, and 0%

respectively). Seven of the adverse events associated with the investigational product were severe, in 3 it was necessary to suspend the investigational product administration, group brimonidine/brinzolamide: allergic conjunctivitis, fatigue, blurry vision. Brinzolamide group: 2 cases of blurry vision. Brimonidine group: 1 case of eye allergy and atopic dermatitis.

Overall, 87 patients were removed from the study due to investigational products-related adverse events: 43 (9.9%) patients in the combination therapy group, 10 (2.2%) patients in the brinzolamide group (2.2%) and 34 (7.5%) subjects of brimonidine group. As can be seen, the incidence of investigational product-related adverse events was slightly greater in the group exposed to combination therapy that in those patients who received brinzolamide or brimonidine as monotherapy.[34]

Baiza L et al., conducted a study which evaluated the safety profile of timolol 0.5%/dorzolamide 2%/brimonidine ophthalmic solution versus timolol/dorzolamide ophthalmic solution in patients with primary open-angle glaucoma or intraocular hypertension receiving 1 drop BID of investigational product and drug comparator for 6 months.

Results: the study involved 112 patients divided into 2 groups of study, 56 subjects per pharmacological intervention. In terms of the safety profile: there were no significant differences in visual acuity, hyperemia, stains, visual fields, symptomatology (eye pain, burning, foreign body sensation and photophobia) between the study groups. Group exposed to dorzolamide/timolol/brimonidine: 2/56 (3.5%) subjects reported at least 1 adverse event. In the group receiving timolol/dorzolamide 6/56 subjects showed at least 1 adverse event. None of the events led to the withdrawal of any patient. The investigational group concluded that both the investigational product and the drug comparator were safe and well tolerated. A better safety profile was seen with the triple combination. (**¡Error! No se encuentra el origen de la referencia.**) [35]

In another study conducted by the research team of *Baiza L, et al* it was determined the safety profile of the fixed combination therapy in ophthalmic solution, consisting of 0.5%/brimonidine 0.02%/dorzolamide 2% versus one solution composed of timolol 0.5%/brimonidine 0.2% in patients with primary open-angle glaucoma and/or ocular hypertension who were divided into 2 study groups, which received 1 drop of the drug comparator or investigational product BID during 3 months. Results: 112 subjects participated, 56 patients per group. During the development of the study any participant presented some serious adverse event. From all participants, only 8 expressed having presented at least 1 non-serious adverse event. 6 subjects were removed from the study due to the development of adverse events. 2 people with ocular burning, one with light intensity and another one with moderate intensity, severe itching in one subject, foreign body sensation of slight intensity in one participant, red eye of severe intensity in another patient, severe dizziness in another patient, mild headache in other participant and bradycardia of mild intensity in one more.

Adverse event	Brimonidine/brinzolamide N = 435 n (%)	Brinzolamide: N = 460 n (%)	Brimonidine: N = 455 n (%)
Ocular			
Blurry vision.	23 (5.3%)	30 (6.5%)	1 (0.2%)
Eye irritation	18 (4.1%)	6 (1.3%)	10 (2.2%)
Eye allergy	11 (2.5%)	0 (0%)	5 (1.1%)
Ocular hyperemia	9 (2.1%)	3 (0.7%)	15 (3.3%)
Eye pain	9 (2.1%)	8 (1.7%)	5 (1.1%)
Allergic conjunctivitis	8 (1.8%)	2 (0.4%)	7 (1.5%)
Eye pruritus	7 (1.6%)	5 (1.1%)	3 (0.7%)
Conjunctival hyperemia	7 (1.6%)	5 (1.1%)	5 (1.1%)
Dry eye	6 (1.4%)	4 (0.9%)	7 (1.5%)
Conjunctivitis	6 (1.4%)	1 (0.2%)	8 (1.8%)
Foreign body sensation	5 (1.1%)	3 (0.7%)	2 (0.4%)
Non-ocular			
Dysgeusia	17 (3.9%)	38 (8.3%)	1 (0.2%)
Dry mouth	13 (3.0%)	0 (0%)	11 (2.4%)
Fatigue	3 (0.7%)	0 (0%)	6 (1.3%)

Table 3. Adverse events in the study by Realini

Adverse event	Brimonidine/timolol/dorzolamide group N = 56	Group dorzolamide/timolol N = 56
Eye pain	1	-
Eye pain and burning	1	-
Eye pain and headache	-	1
Keratitis and eye pain	-	2
Keratitis and chemosis	-	1
Keratitis and burning	-	1
Burning	-	1

Subjects who reported at least 1 adverse event during their participation in the study.

Table 4. Adverse events in the study by Baiza 2009

Adverse event	Brimonidine/timolol/dorzolamide group N = 56	Group timolol/brimonidine N = 56
Eye burning	1	1
Itching	1	-
Foreign body sensation	1	-
Red eye	-	1
Dizziness	-	1
Headache	-	1
Bradycardia	-	1

Subjects who reported at least 1 adverse event during their participation in the study.

Table 5. Adverse events in the study by Baiza 2012

The research team concluded that the safety profile of brimonidine, timolol and dorzolamide ophthalmic solution was better, under that there were only 3 events compared to the 5 reported in patients who received the dual therapy. However, both therapies were considered safe. [36] (**¡Error! No se encuentra el origen de la referencia.**)

Garcia A, et al., reported the safety profile by developing a cross-over clinical trial, 2 periods, 2 sequences involving patients with primary open-angle glaucoma or ocular hypertension who previously received ophthalmic solution in fixed combination of bimatoprost 0.03%/timolol 0.5% or ophthalmic solution in fixed combination of brimonidine 0.2%/timolol 0.5%/dorzolamide 2% at a dosage of 1 drop 2 times a day for 3 months; in each period, for subjects exposed to triple therapy and a dose of 1 drop once a day for 3 months; in each period, for patients with double combined therapy.

Results: Few adverse events were reported by participants throughout the study. The most common event was hyperemia and was the only event associated with the investigational drug [37].

As it could be observed in the **¡Error! No se encuentra el origen de la referencia. Adverse events in the study by García,** it is shown that the event took place in the whole population evaluated during the course of the study, however, or refers to the type of intervention that the participant was receiving when the event that led to the withdrawal of the patient happened.

Adverse reactions associated with the timolol maleate 0.5% at eye level can be: transient burning, decreased corneal sensitivity, allergic blepharoconjunctivitis, superficial punctate keratitis, conjunctival hyperemia, keratoconjunctivitis sicca, ptosis, diplopia, blurry vision and refractive changes (due to, in some cases, the suspension of treatment with miotics). At the level of the central nervous system: headache, dizziness, lethargy, confusion, hallucinations, fatigue and depression. Cardiovascular: Systemic arterial hypotension, bradycardia, arrhythmia, atrioventricular heart block, cardiac arrest, congestive heart failure, syncope, stroke, tachycardia and cerebral ischemia; the latter have been most common in older people or people with previous cardiovascular alterations. Adverse reactions to respiratory level: bronchial spasm, (especially in subjects with a history of broncoespasticos disorders), respiratory failure and shortness of breath. Other reactions: worsening of the myasthenia gravis, alopecia, nail pigmentary changes, nausea, localized and widespread rash

skin, urticaria, asthenia, sexual dysfunction, including impotence, decrease of the libido and reduced ejaculation; hyperkalaemia, masking of symptoms of hypoglycemia in patients with diabetes insulin, diarrhoea and paraesthesia. [38]

Adverse reactions reported frequently with the use of brimonidine tartrate 0.2% are: dry mouth sensation, drowsiness, fatigue, cardiopulmonary disorders. At eye level: hyperemia, burning, blepharoconjunctivitis and allergic conjunctivitis, acute eye conjunctivitis, transient miosis in people sensitive to the formula.[39]

Adverse event	Brimonidine/timolol/dorzolamide group N = 78	Group timolol/bimatoprost N = 78
Vitreous hemorrhage	1	
Pruritus	1	
Severe hyperemia*	1	2
Sensation of foreign body and conjunctival edema *	-	1
Serious eyelid edema	1	
Visual field reduction*	1	-
Allergy	1	
Significantly reduced visual acuity	1	
Blurry vision.	1	
Not specified*	1	-

Events reported in patients during the 6 month study. Overall 12 patients showed at least 1 adverse event, 6 of them had to withdraw from the research protocol because of the severity and seriousness of the event. *Subjects withdrawn from the study.

Table 6. Adverse events in the study by García

Dorzolamide hydrochloride 2% is generally well tolerated. Adverse effects reported with greater frequency have been transient burning eye, taste distortion, corneal erosion, conjunctival injection, blurry vision, tearing and eye itching. [40]

5.3.3 Preservative use in ocular hypotensive medications

Preservatives are used in order to inhibit microbial growth and to suppress the biodegradation of drugs. [41] Throughout history a variety of preservatives have been used. Chronic use of drugs has been linked to irritation and alterations of the ocular surface; it has been established that preservatives have the same adverse events, which are attached to the active principle that preserve. [42]

Preservatives are divided into detergents and oxidative, and a more recent version of oxidatives named ionic buffered. [41] Historically, BAK is the most widely used preservative, it is a class of detergents, and its concentration in ocular hypotensive drugs ranges from 0.004-0.02%. The reasons of their popularity are its broad action spectrum and the familiarity with the industry.

While its effectiveness is well recognized, there are numerous studies documenting the harmful effects of BAK. BAK, at 0.05-0.1% concentration, induces necrosis, and at 0.01%, apoptosis. Its effects are cumulative and are more severe at higher concentrations and more frequent exhibitions. [41] [43] It has been proposed that BAK is responsible for changes in the conjunctival surface identified by impression cytology, as the decrease in the density of calyceal cells.[44] [45]

5.4 Justification

During the treatment of a patient with glaucoma, it is possible that, at some point, 2 or more drugs are required to achieve the IOP desired control. Fixed combinations offer many advantages, including a simplified regime that can lead to a better attachment, a convenience for patient as a result of using fewer bottles and instillations, a less exposure to preservatives that could potentially damage the ocular surface, eliminating the washing effect and finally a cost-savings in the cost of therapy.

In Mexico, since 2007, and recently in other Latin American countries, such as Bolivia, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Panama, Peru, Dominican Republic and Uruguay, is commercially available Krytantek Ofteno®, developed by Laboratorios Sophia, S.A. de C.V., which is the fixed combination of timolol 0.5%/brimonidine 0.2%/dorzolamide 2% ophthalmic solution. During these years, Krytantek Ofteno® has shown its effectiveness in clinical practice of ophthalmologists who use it, and has been known for its safety profile by pharmacovigilance systems.

PRO-122 is a preservative-free Krytantek Ofteno® reformulation, and there are currently no reports of the effectiveness of this fixed triple combination compared to its components applied concomitantly. A clinical study of non-inferiority is the more appropriate tool to assess this information.

5.5 Objectives and hypothesis

5.5.1 General objective

To evaluate the non-inferiority in the ocular pressure decrease of the preservative-free ophtalmic solution PRO-122, manufactured by Laboratorios Sophia, S.A. de C.V., versus concomitant therapy in subjects with uncontrolled POAG and/or IOP.

5.5.2. Specific objectives

- To compare the effectiveness of reducing the IOP in the ophthalmic solution PRO-122 versus reducing the IOP in Krytantek Ofteno® and concomitant therapy.
- Compare the safety of the ophthalmic solution PRO-122 versus Krytantek Ofteno® and the concomitant therapy.
- Compare the safety of the ophthalmic solution PRO-122 versus Krytantek Ofteno® and the concomitant therapy by the adverse events.
- Compare the safety of the ophthalmic solution PRO-122 versus Krytantek Ofteno® and the concomitant therapy by the ocular surface integrity.
- Analyze the tolerability of the ophthalmic solution PRO-122 versus Krytantek Ofteno® and the concomitant therapy.

5.5.3 Hypothesis

H_0: The mean (average) value of the IOP final absolute reduction in the experimental group (PRO-122) is lower, considering a lower limit of 1 mmHg, compared to the IOP mean absolute reduction of the standard group (concomitant therapy).

H_a: The mean (average) value of the IOP final absolute reduction in the experimental group (PRO-122) is not lower, considering a lower limit of 1 mmHg, compared to the IOP mean absolute reduction of the standard group (concomitant therapy).

> *Note*: Absolute reduction means the total decrease in mmHg of the PIO expressed in positive numbers. It is calculated as the subtraction of the IOP value on the final visit to the baseline visit (basal IOP - final IOP)

5.6 Study plan and design

A non-inferiority, double-blind, randomized, controlled, parallel, phase-III clinical trial. Patients participating have primary open-angle glaucoma and/or ocular hypertension without control with dual-fixed combined therapy or concomitant dual therapy, who will be randomly assigned to 3 groups of hypotensive pharmacological intervention. A group will receive the test formulation PRO-122 + approved placebo (2 multidose dropper bottle), another group will be exposed to Krytantek Ofteno® + approved placebo (2 multi-dose dropper bottle) and finally a group will receive concomitantly 3 multidose dropper bottle, which contain individually the active agents: brimonidine 0.02%, dorzolamide 2% and timolol 0.5%.

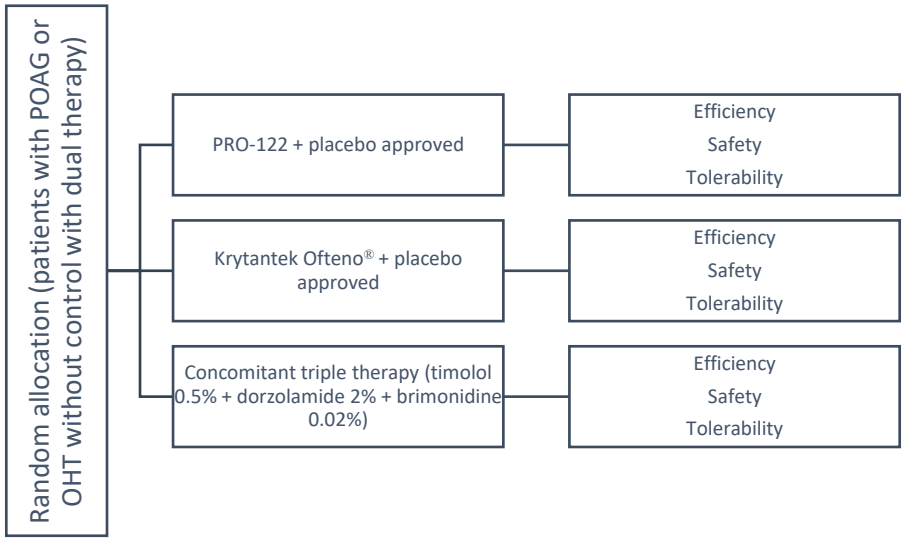


Figure 1. Study design

5.6.1 Discussion of the study design

In clinical investigation, it is common to propose tests in order to assess the efficacy of an investigational product against a drug reference or active control, in order to show that the new investigational product is not inferior from the clinical or statistical perspective to a medication commercially available. Studies with objectives such as the former are referred to as trials of unilateral equivalence or non-inferiority. The most important criterion of this kind of research designs is the *a priori* definition of a quantity or value (of the variable of primary outcome) that marks a clinical or statistically acceptable difference between the treatments being compared. Like this, the hypothesis testing planning, results in the selection of a proper non-inferiority limit, which can be obtained from the reference drug product superiority studies results versus placebo.

The clinical trial is the ideal model to evaluate the effectiveness of two interventions, allows to obtain evidence of greater quality between the different types of research. The features of randomization and double-blind allow to avoid bias (selection, assessment, etc.) which cannot be avoided with other models. The controlled and parallel-group characteristic allows to distinguish the effects of the interventions alone.

In the present research is determined the limit of non-inferiority as the value obtained of the difference of the intraocular pressure (mean baseline number minus mean final number of absolute reduction, expressed in positive numbers) - 1, i.e. delta of intraocular pressure -1 will be the limit of unilateral equivalence or of non-inferiority to establish the compliance of patients exposed to the investigational product PRO-122. When exceeding that limit it does not comply with the assumption of not be lower with regard to hypotensive drugs administered concomitantly. To understand the previously said, it is important to consider the following: it is known, from others prospective studies, that by each millimeter of mercury increasing in the intraocular pressure in a patient with POAG, the risk of disease progression increased in a 5%, this is why the main goal of treatment is reduction. [46]

The proposed clinical trial aims to evaluate the profile of efficacy, safety and tolerability of a fixed combined formulation, without benzalkonium chloride, formed by brimonidine 0.2%/dorzolamide 2%/timolol 0.5% and to compare it against the profile of safety, tolerability and efficacy of the concomitant administration of active agents in individual pharmaceutical forms. The addition of a group of patients who were exposed to the reference drug product Krytantek Ofteno®, which is dispensed equally as a fixed combination therapy consisting of the same active agents and with the use of benzalkonium chloride in the formulation was judged as suitable.

6. Material and methods. Participants, interventions and variables

6.1 Study center

This study will take place in ophthalmology practices properly equipped and registered for their proper functioning. According to the needs of the sponsor these may be either private or public, be attached to a hospital or practice or be independent.

This is a multicenter study intended to be performed in Argentina, Mexico and Colombia.

6.1.2 Center organization

Each study center will have a principal investigator (PI). The PI is the specialist in ophthalmology, leader of the clinical study.

The PI is responsible of conforming a multidisciplinary research team to run the clinical study according to the protocol, under scientific guidance. IP prerogative is the organization design from its core and the selection of the staff who shall perform such functions. However, the minimum organization of research team requested by the sponsor requires the subinvestigator, studies coordinator and pharmacist.

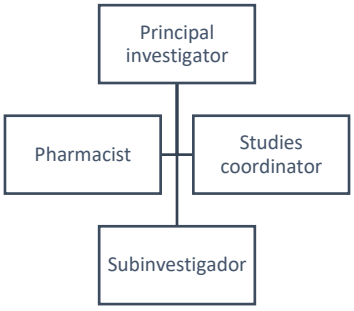


Figure 2. Minimal organization of the Center

Every person appointed by the PI, under their responsibility, a part of the follow-up of the study (coinvestigador, subinvestigador, nurse, etc.) or a study participation specific function (pharmaceutical, administrative auxiliary, study coordinator, etc.) must appear in the form "Delegation of Responsibilities".

The "delegation of responsibilities" and "Flow chart of the Center" will be delivered to the sponsor before the start of the study and updated if members or their responsibilities change.

6.1.3 Documentation to be delivered to the sponsor

PI must deliver it to the sponsor before starting the study:

- Updated *curriculum vitae* in Spanish, dated and signed (maximum 10 pages), of PI and staff of the organization chart.

- Copy of academic certificates of PI (degree diplomas in ophthalmology; professional ID or equivalent corresponding to the country)

- Copies of academic certificates of maximum grade achieved, of each member of the research team that support their capacity to play their corresponding roles.

- Copy of notice of performance or equivalent issued by the corresponding regulatory entity (if applicable)

- Certificate of current good clinical practices. In case the certificate of the issuing institution does not specify the validity date, the date of issue of the certificate shall not exceed one year

6.1.3 Center closure

The closing of the center will be done once the last visit of the last subject included previously agreed between the sponsor and the PI has been performed. The closing process will be performed according to the in-house standard operation procedures of the sponsor.

The premature closure of a study center is the prerogative of the sponsor, and it should be informed to the PI the reasons for the closure.

6.2 Elegibility criteria

6.2.1 Inclusion criteria

- Signed informed consent
- Age ≥ 18 years
- Both sexes
- Women of childbearing age with birth control method
- Diagnosis of POAG (according to the Guidelines of the Preferred Practice Pattern of the American Academy of Ophthalmology) or OHT
- IOP not controlled with dual therapy according to the investigator judgement.
- IOP on the selection visit at 9 am, after the washing period, from 21 - 36 mmHg in at least one eye.

6.2.2 Exclusion criteria

6.2.2.1 General criteria

- Pregnant, breastfeeding or planning to get pregnant women.

- Women of childbearing age and who do not intake a hormonal contraceptive method, IUD or bilateral tubal obstruction.
- Participation in another clinical research study ≤ 30 days before the screening visit.
- People who cannot comply with their attendance at appointments or with all the Protocol requirements

6.2.2.2 Medical and therapeutic criteria

- Anterior chamber angle grade <2 of Shaffer rating.
- Excavation of optic nerve > 0.80 horizontal or vertical (ratio cup-disc)
- Serious loss of central visual field in any eye (sensitivity ≤10 dB in ≥ 2 of 4 points of the visual field test close to the fixation point)
- People not able to safely suspend ocular hypotensives drug products for the washout period according to the IP judgement.
- Chronic, recurrent, or active ocular inflammatory diseases (e.g. uveitis, scleritis, keratitis, herpetic) in any eye.
- Eye trauma ≤ 6 months prior to the study
- Eye infection / inflammation ≤ 3 months prior to the study
- Clinically significant or progressive retinal disease (e.g. degenerations, diabetic retinopathy, retinal detachment)
- CV 20/200 or worse in any of the eyes.
- Subject with only one eye
- Eye diseases that contraindicate the use of BB, AA or IAC
- Intraocular surgery ≤ 6 months prior to the study
- Laser intraocular surgery ≤ 3 months prior to the study
- Any abnormality preventing reliable applanation tonometry
- Unstable or uncontrolled cardiovascular disease
- Chronic pulmonary disease (e.g. bronchial asthma)
- Any condition or illness that do not fit the subject for the study according to the PI judgment.
- Use of high doses of salicylate (1 g daily) ≤ 4 weeks before the eligibility visit
- In treatment with psychotropic medications that increase the adrenergic response
- Known hypersensitivity to BB medications (e.g. timolol), AA (e.g. brimonidine) and IAC (e.g. dorzolamide), sulfonamide derivatives, or any of the components of the study drugs
- Concomitant use of MAO inhibitors
- Systemic or topical use of corticosteroids

6.2.3 Elimination criteria

- Subject decision. The subject can decide unilaterally to withdraw the study in any moment, and should inform it to the PI.
- Pregnancy (considered an SAE)
- Presence of a SAE
- Decrease of IOP in less than 20%, compared to the baseline, for consider it an AE by lack of efficiency
- Decision of the investigator:
    - By an AE that, according to the IP judgement, threatens the subject health and requires the prescription of a drug product not authorized in the Protocol
- Any deviation to the Protocol that affects the subject safety
- Adherence lower than 40% in some of the visits
- Subject that does not attend to two consecutive visits

6.2.4 Subject identification

Patients of the study will be identified by a number and their name initials.

The subject's initials will be the first letter of the name, followed of the first letter of the first last name and the first letter of the second last name, obtaining a maximum of three letters; in case the person has two names or a compound last name it will always be used the first letter of the first name or compound last name.

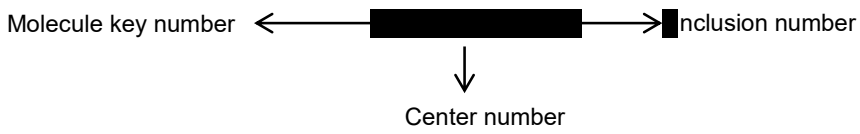
Example:

1. Adolfo Daniel Mercado Carrizalez
    a. Initials: AMC
2. Juan De la Torre Orozco
    a. Initials: JDO
3. Luis Carlos Pérez-Gómez Ramírez
    a. Initials LPR

In the screening stage you will assign a participant number consecutively, using 3 consecutive digits. Once the subject has been selected, it will be assigned with a number with which will be identified during all the study. This code shall consist of eight numbers in the following order from left to right:

- three numbers of the study molecule according to the denomination by the sponsor.
- two digits corresponding to the number of investigational center
- three digits of the consecutive number of the inclusion assigned in the investigational center.

Example:



6.3 Intervention

6.3.1 Given treatments

Treatments will be administered twice a day, at 9:00 am (09:00 hours) and at 9:00 pm (21:00 hours). In all treatment groups 3 bottles will be given to each subject, these will be tagged with a random assignment number and the management sequence letters: A, B and C (see 7.2 Blinding mechanism), the subject must start with the application of bottle A, wait 5 minutes before applying the contents of bottle B, after this second application wait another 5 minutes for the application of bottle C.

6.3.1.1 Study treatment

- PRO-122
    - Drug substances: Timolol 5 mg/mL, brimonidine 2 mg/mL and dorzolamide 20 mg/mL. See **¡Error! No se encuentra el origen de la referencia.** [48]
    - Pharmaceutical form: Conservative-free ophthalmic solution
    - Dosage form: 5 mL multidose dropper bottle
    - Made by: Laboratorios Sophia, S.A. de C.V.
    - Posology: 1 drop every 12 hours for 90 days
    - Description of the solution: clear, visibly particle free solution, with a pH range of 5.4 to 5.9 and a slightly yellow color

- Description of the container: low density polyethylene white bottle with 5mL capacity. Assembled high density polyethylene container-closure system with a silicone and low density polyethylene valves system, which allows to preserve the sterile solution without any additives.

- Placebo
  - Two pieces of approved placebo. Administered in 2 multidose dropper bottles.
  - Posology: 1 drop of each dropper bottle every 12 hours for 90 days

Type of agent	Amount mg/mL	Function
Dorzolamide hydrochloride	22.25*	Drug substance
Timolol maleate	6.80+	Drug substance
Brimonidine tartrate	2.00	Drug substance
Hydroxypropyl methylcellulose	Not shown	Additive
Mannitol	Not shown	Additive
Citric acid monohydrate	Not shown	Additive
Sodium citrate dihydrate	Not shown	Additive
Water for injection q.s.	1.00	Vehicle

Investigational product PRO-122 qualitative and quantitative formulation. Concentration of those drug substances, as well as the substances functioning as additives, is shown.
(*) Equivalent to dorzolamide 20.00 mg/mL (+) equivalent to timolol 5.00 mg/mL

Table 7. PRO-122 qualitative and quantitative formulation

6.3.1.2 Reference treatment

6.3.1.2.1 Krytantek Ofteno Group

- Krytantek Ofteno®.
  - Drug substances: Timolol 5 mg/mL, brimonidine 2 mg/mL and dorzolamide 20 mg/mL. See **Table 8: Qualitative/quantitative formulation of Krytantek Ofteno®** [49]
  - Pharmaceutical form: Ophthalmic solution
  - Made by: Laboratorios Sophia, S.A. de C.V.
  - Posology: 1 drop every 12 hours for 90 days
  - Description of the solution: clear, visibly particle free, slightly yellow solution
  - Package description: 5 m multidose dropper bottle.
- Placebo
  - Two pieces of approved placebo. Administered in 2 multidose dropper bottles.
  - Posology: 1 drop of each dropper bottle every 12 hours for 90 days

6.3.1.2.2 Concomitant triple therapy group

  - Imot Ofteho®
    - Drug substance: Timolol 5 mg/mL
    - Pharmaceutical form: Ophthalmic solution
    - Made by: Laboratorios Sophia, S.A. de C.V.

- Alphagan®
  - Drug substance: Brimonidine 2 mg/mL
  - Pharmaceutical form: Ophthalmic solution
  - Made by: Allergan, Inc.
- Trusopt®
  - Drug substance: Dorzolamide 20 mg/mL
  - Pharmaceutical form: Ophthalmic solution
  - Made by: Merck Sharp & Dohme Corp.
- Posology: 1 drop every 12 hours for 90 days

Agent type	Quantity in mg/mL	Function
Dorzolamide hydrochloride	22.25*	Active ingredient
Timolol maleate	6.80+	
Brimonidine tartrate	2.00	
Polyethylene glycol Monostearate	Not available	Additive
Manitol	Not available	Additive
Sodium Chloride	Not available	Additive
Sodium Tetraborate Decahydrate	Not available	Additive
Benzalkonium chloride	Not available	Additive
Water for injection c.b.p	1.00	Vehicle

Qualitative/quantitative formulation of test drug Krytantek Ofteno® (Reg. No. 074M2077 SSA IV). Concentrations of active ingredients and additive substances.

(*) Equivalent to 20.00 mg/mL of Dorzolamide (+) Equivalent to 5.00 mg/mL of Timolol

Table 8. Qualitative/quantitative formulation of Krytantek Ofteno®

6.3.2 Strategies to improve the adherence and procedure for monitoring the adherence

Strategies:

- During visit 2, visit 3 and the final visit, the test subject must return the assigned bottle in order to verify the treatment adherence by checking the weight of the bottle returned to the site.
- In each visit the PI must remind the test subject of the importance of following the assigned treatment and must ask whether he/she had any difficulties in following the instructions and, if necessary, must retrain the subject to apply the test drug.
- The PI or a designee must send emails to subjects in order to remind them of the treatment adherence and the importance of it. The content of such emails must be previously approved by the Institutional Review Board.
- Use of a printed reminder.
- Use of the subject diary.

Procedure for monitoring the adherence:

- Procedure for the investigation center: before supplying the test subject with the drug, the pharmacist must weigh the bottles to be supplied. The weighing must be performed once again after the subject had returned the drug. Considerations to take into account:
    - Pharmacist must use the scale provided by the sponsor.
    - He/she must put the bottle in the center of the scale and check the weights.
    - He/she must remove the bottle from the scale and put it back again in order to verify that the measurement taken is the same as the previous one. If different, he/she must weight it again and record the average of three measurements.
    - He/she must record the result in a log provided especially for this purpose.

Adherence must be assessed based on the weight of an empty bottle, the weight of a single drop, the weight of the filled bottle, the total amount of drops to be applied during the intervention period and the total weight of all applied drops. This simplified formula must be used:

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

Where:
Ad = adherence
P_i = weight of the bottle initially supplied to the subject
P_f = weight of the bottle returned by the subject.
P_T = weight of the dose indicated for the intervention.

$$P_T = (P_g)G$$

Where:

P_g= weight of the intervention drop, determined by the R&D department.

G = number of drops indicated for the intervention.

Adherence is assessed at every visit with the return of bottles by test subjects. Results will allow the PI to determine if the subject continues being part of the study, as per the exclusion criteria.

Adherence must be assessed by reviewing the subject diary. This formula must be used:

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

Where:
Ad = Adherence
A_r = Recorded applications
A_i = Applications indicated for the intervention

Final (global) adherence must be determined based on the adherence average of each visit.

If a subject fails to return 2 to 4 bottles at least, his/her data must not be taken into account for the calculation of the final adherence.

6.3.3 Allowed and prohibited concomitant treatments and interventions before and after the study

Subjects duly enrolled to the study and complying with all eligibility criteria area will be able to continue their systemic treatments for baseline diseases. If during the study, they need to add a new allowed drug, they will be able to do so. All used concomitant drugs must be duly recorded in the clinical file notes and in the corresponding section.

Allowed drugs:

– Ophthalmics:

All ophthalmic allowed drugs during the study must be applied at least 10 minutes after the last application of the study/reference treatments. This procedure has the aim of avoiding treatment interactions in the tear film, based on tear flow rate and physiological tear volume. [18]

- Tetracaine 0.5%
- Tropicamide 0.8%/ phenylephrine 5%
- Any antibiotic
- Ocular lubricants

- Other drugs not ophthalmically applied:

- The use of drugs whose effect could have an effect on some of the efficacy, safety and tolerability parameters of this investigation protocol must be notified to the clinical monitor or the sponsor scientific committee in order to determine the convenience of the subject admission, stay or withdrawal.

Prohibited drugs:

– Any other ophthalmic drug that is not part of the list of allowed drugs.
– Systemic carbonic anhydrase inhibitors (e.g., acetazolamide)

6.3.3.1 Washout period

Washout periods for ocular hypotensive drugs must be: ≥5 days for topical miotic agents and topical/oral carbonic anhydrase inhibitors; ≥14 days for AA and ≥28 days for prostaglandin analogues and BB (See **Table 9, Washout period**), based on the periods used from previous studies. [50] For combined administrations, the longest washout period for individual components must be used. At the discretion of the PI, patients will be moved to a drug requiring a shorter washout period.

Drug Group	Example	Washout period (days)
Miotics (topical agents)	Pilocarpine	≥5
Carbonic anhydrase inhibitors (oral and topical agents)	Acetazolamide/ Brinzolamide, Dorzolamide	≥5
α-Agonist	Brimonidine, Apraclonidine	≥14
Prostaglandin analogues	Travoprost, Latanoprost	≥28
β-blockers	Timolol, Betaxolol	≥28

Table 9. Washout Period

6.3.4 Treatment management

Treatments will be provided by Laboratorios Sophia, S.A. de C.V. to each investigation center. Drugs must be previously labeled, reconciled and weighed. The PI or some designated member of his/her team is responsible for the treatment management.

6.3.4.1 Supply and reception

The sponsor has the responsibility for supplying the study drugs in each investigation center as per the internal procedures. Drugs must be supplied in closed boxes through delivery service or directly by the sponsor personnel to the address of the investigation center as per the study plan.

Reception will be exclusively performed by the investigation center team, including the PI. The primary packaging (box) has to be in good state. Any alteration or imperfection which, at his/her discretion, might compromise the integrity of its contents must be reported to the sponsor. If the package does not show significant imperfections, he/she must proceed to open it.

Inside he/she will find the goods received note and a temperature/humidity data logger. He/she will also check that the recorded temperature and humidity meet the specifications for transport and storage conditions (see 6.3.4.2, “Storage”). Then, he/she must check the contents (treatment drugs) against the contents stated in the document. Provided the contents match the information stated in the document, he/she must sign the goods received note and must mail it to the sponsor. Otherwise, he/she will notify the sponsor of the given discrepancies.

At the study center, personnel assigned by the PI must supply subjects with their corresponding treatments by giving them quantities enough to cover the treatment period. Treatment drug supply must be performed by stages: at baseline and in visit 2 and visit 3.

6.3.4.2 Storage

Drug must be stored in a safe area with restricted access. Storage temperature must be at room temperature, no greater than 30 °Celsius.

The investigation center is responsible for recording, in the form supplied by the sponsor, the temperature and humidity reported by the data logger. The record must include current temperature and humidity, and minimum and maximum recorded values for both of them. This must be done on a daily basis (on business days) and as long as the protocol remains effective.

The clinical monitor must compare the data against the data reported by the data logger.

6.3.4.3 Returns

Test subjects must return the drugs to the personnel assigned by the PI in the treatment centers at visit 2, visit 3 and the final visit.

Returns will be performed by the investigation center personnel whenever the sponsor indicates so. Before returning the drugs to the investigation center, the remaining amount of drug must be counted and checked against the initial amount in order to produce an inventory that serves in the filling of the drug return form.

6.4 Outcome variables

6.4.1 Safety variables

6.4.1.1 Primary outcome variables

Best corrected visual acuity

Cup-to-disc ratio

Visual fields determined by computerized perimetry

Ocular surface integrity

-Conjunctival hyperemia

- Chemosis

- Fluorescein staining

Central corneal thickness

Adverse events

Goblet cell density

6.4.1.2 Tolerability variables

Ocular comfort index

6.4.2 Efficacy variables

6.4.2.1 Primary outcome variables

IOP lowering

6.4.2.2 Secondary outcome variables

Not applicable

6.4.3 Methods and gradings to be used in the measurement of variables

Variable	Unit	Symbol	Type	Measurement method
Best corrected best corrected visual acuity	Decimals	---	Continuous	Snellen chart
Visual fields	Decibels	dB	Continuous	Computerized perimetry
Goblet cell density	Cells per square millimeter	cel/mm^2	Continuous	Impression cytology
Central corneal thickness	Micra	µm	Continuous	Ultrasound pachymetry
Conjunctival hyperemia	Normal/ very mild/ mild/ moderate/ severe	---	Ordinal	Direct observation (biomicroscopy)
Ocular comfort index	Points	---	Discrete	Questionnaire
Presence of adverse events	Number of cases	n	Discrete	Count of cases
Intraocular pressure	Millimeters of mercury	mmHg	Continuous	Goldman applanation tonometry
Chemosis	Presence/ absence	---	Nominal	Direct observation (biomicroscopy)
Cup-to-disc ratio	Decimals	---	Continuous	Direct observation (biomicroscopy)
Surface staining	Points	---	Discrete	Direct observation (biomicroscopy)

Table 10. Method for the measurement of variables

The following are methods and gradings used in the measurement of variables:

6.4.3.1 Visual fields

Visual field refers to the perception by the visual cortex of objects and light sources in a given moment through visual fixation. Perception in the cortex implies that the object or light source was visually processed through retinal stimuli. The visual field comprise a three-dimensional cone (Traquair's Island of vision) having its apex at the nodal point of the eye and its base at infinity. [51] The peripheral nature of the visual field covers approximately 60 °in the top section, 60 ° in the nasal section, 80 ° in the low section and 90 ° in the temporal section. The blind point is located in the temporal section between 10° and 20°.

Perimetry represents a psychophysical test that aims to determine the peripheral limits of the visual field of any given subject.

Humphrey's perimetry is an automated test whose software includes programming for standardized strategies with predefined intervals (e.g. 30-2, 24-2, 10-2). The full threshold strategy represents the reference standard for glaucoma testing. Nevertheless, the Swedish Interactive Thresholding Algorithm (SITA) can replace such strategy as it is faster and more likeable and sensitive. [52]

Visual fields must be obtained with an automated perimeter (Humphrey's perimeter) by performing white on white tests using the 24-2 SITA-standard. All perimetry results included in the study will be deemed as reliable if they show at least 20% of fixation loss, false positives, and false negatives. [53] Mean deviation, a global measure of visual field loss, and the standard deviation of model, a measure of focal loss or field variability, must be recorded in the CRF.

6.4.3.2 Best corrected visual acuity

Visual acuity represents a visual function test. Spatial visual acuity is the capacity to distinguish separate elements from an object and identify them as a whole. It is quantified as the minimum angle of separation (located in the nodal point of the eye) between two objects that allows the perception as separate objects.

The Snellen notation is described as the distance used in the test, divided by the distance at which letters subtend an angle of 5 minutes of arc. Therefore, with a distance of 6 meters a 6/6 letter (20/20) equals 5 minutes of arc, a 6/12 letter (20/40) equals 10 minutes and a 6/60 letter (20/200) equals 50 minutes. Snellen fractions can also be expressed as decimal numbers (i.e. 20/20 = 1 and 20/40 = 0.5). [52]

Visual acuity represents a basal assessment for patients without previous refractive corrections and it involves a Snellen chart located in a place with sufficient amount of natural/artificial light and at a distance of 3 meters from the subject to be examined. Visual acuity is assessed in both eyes, beginning with the right eye. The subject is asked to keep both eyes open and, having the left eye covered with an occluder, the subject must be asked to read out loud the lines that the evaluator points. The line having the smallest letters which the subject still is able to read must be recorded by the evaluator in the clinical file as visual acuity fractions. The left eye must be evaluated in the same way.

Afterwards, the best refractive corrections must be performed and the test must be performed once again using the obtained refraction. Results must be recorded in the clinical file and in the CRF as fractions of best corrected visual acuity (the CRF must include decimal numbers.) By definition, the best corrected visual acuity cannot be inferior to the visual acuity.

6.4.3.3 Goblet cell density

The density of goblet cells in the conjunctiva can reflect the seriousness of ocular surface alterations [54]. Normal density has been determined as superior to 500 cells/mm^2. [55] Impression cytology refers to the application of a cellulose acetate on the ocular surface in order to remove epithelium surface layers; cells, once removed, can be subject to histological, immunohistological and molecular analyses. [56]

Impression cytology in the conjunctiva must be performed by the investigator using cellulose acetate filter millicell and the device supplied by the sponsor especially designed for this purpose. Such device has a circular cell of approximately 10 mm of diameter. Once the ocular surface be anesthetized with topical tetracaine, the subject must be asked to expose the temporal ocular surface to be evaluated by the investigator and the latter will gently press the cell against the temporal conjunctiva (2 to 3 mm from the corneal-scleral limbus) for 5 seconds and remove the cell by peeling (**Figure 1. Impression cytology**). Immediately after, the investigator must adhere it with diethyl ether in spray by releasing one or more discharges 15 cm away. The investigator must contact the courier designated by the sponsor in order to send the samples for histopathological analyses.

Ocular surface staining, when required, must be performed after this procedure.

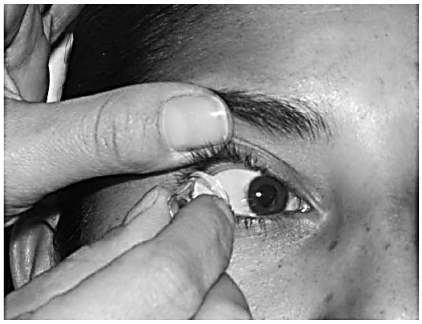


Figure 3. Impression cytology

6.4.3.4 Ocular comfort index

The ocular comfort index is measured by a questionnaire designed for the measurement of the ocular surface irritation by Rasch analysis in order to produce estimates in a lineal interval scale (0-100 scale). Like ocular surface disease index, ocular comfort index (OCI) evaluates symptoms. OCI comprises eight items (one positive and seven negative) that focus on the discomfort associated with ocular surface diseases. Each of these questions have two parts, which separately establish the frequency and severity of symptoms. [57] See annex 13.1 Ocular comfort index.

The evaluator must supply the subject with the questionnaire and ask him/her to fill it with calm and without any kind of pressure and/or coercion. The evaluator must be of assistance only if he/she has any difficulty in understanding the questions.

6.4.3.5 Ocular surface integrity

This test is performed in the investigation center by slit lamp microscopy. A complete examination of the anterior segment must be performed and results must be recorded in the clinical file. Lighting techniques must be determined by the PI.

The variables to be recorded in the CRF are the following:

- Conjunctival hyperemia. It is defined as the simplest conjunctiva reaction against any given stimulus. A red appearance derived from variable-intensity vasodilatation of conjunctiva

vessels. Grading must be performed following the Efron scale [58]. See annex 13.2, Efron scale for conjunctival hyperemia.

- Chemosis. It is defined as a conjunctival edema as a result of inflammatory reaction. It is qualified as either present or absent. The evaluator must use a narrow beam of light directed from an angle of 60° and must check whether the separation of the conjunctiva from the sclera is >1/3 of the total height of the palpebral fissure or whether the conjunctiva prolapses the grey line of eyelid. [59]
- Fluorescein staining. A drop of topical anesthetic is instilled in the conjunctival cul de sac. Afterwards, a second drop must be instilled in the edge of the fluorescein strip, allowing it to slip to the cul de sac. It is important to perform the staining in a quick and sequential manner —first the right eye and then the left eye— so that the observed patterns be equally bright. This test must be performed with cobalt blue filter. Grading must be as per the Oxford scale. [60] See annex.

6.4.3.6 Corneal pachymetry

Although the central corneal thickness (CCT) and IOP have an independent effect on the risk of developing glaucoma, such factors do interact between themselves. With the introduction of Goldmann's applanation tonometry, the CCT has since been the potential confounding factor in the measurement of IOP. The CCT must be measured by ultrasound pachymetry. Three measurements must be recorded in the clinical file and the average of them must be noted down in the CRF. Baseline and final pachymetry must be performed by the same evaluator using the same pachymeter. IOP must be readjusted using the Ehlers formula: [61]

$$IOP - \frac{\left[5.0 * (\frac{GCC}{1000} - 0.520)\right]}{0.070}$$

6.4.3.7 Adverse event presence

Adverse events must be handled as per section 9.3, Adverse events.

The PI must record in the corresponding section of the CRF all AEs of study subjects.

6.4.3.8 Intraocular pressure

Tonometry is the objective measurement of the IOP —based must of all on the force necessary to applane the cornea— or the level of corneal indentation produced by a fixed force. Goldman's tonometry is based on the Imbert-Fick law. [52]

Once the eye is instilled with a topical anesthetic, fluorescein tonometry must be performed using a blue cobalt filter (after corneal surface staining test). Two readings are to be taken and recorded in the clinical file. The average must also be recorded in the CRF. Tonometry must performed between 9:00 a.m. and 11:00 a.m. (± 30 minutes), which is the time after which a minimum effect (12 hours post-instillation) and a maximum effect (+2 hours post-instillation) of IOP lowering is reached, respectively. Throughout the treatment, the investigator must make sure that the test subject applies the treatment after the IOP check at 09:00 a.m.

6.4.3.9 Cup-to-disc ratio

Cup-to-disc ratio is measured by slit lamp indirect ophthalmoscopy. Lens is to be chosen at the discretion of the PI. Mydriasis has to be induced by pharmacological means (tropicamide 0.8% / Phenylephrine 5%) in order to perform an accurate examination of the optic disc.

6.4.4 Measurements by stage

6.4.4.1 Measurements taken during screening

- Intraocular pressure
- Best corrected visual acuity
- Cup-to-disc ratio
- Ocular surface integrity, including:
    - Conjunctival hyperemia
    - Chemosis
    - Fluorescein staining

6.4.4.2 Measurements taken at baseline
- Intraocular pressure
- Best corrected visual acuity
- Cup-to-disc ratio
- Visual fields determined by computerized perimetry
- Central corneal thickness determined by pachymetry:
- Ocular surface integrity, including:
    - Conjunctival hyperemia
    - Chemosis
    - Fluorescein staining
- Goblet cell density
- Ocular comfort index

6.4.4.3 Measurements taken during visit 1
- Intraocular pressure
- Best corrected visual acuity
- Ocular surface integrity, including:
    - Conjunctival hyperemia
    - Chemosis
    - Fluorescein staining
- Adverse events

6.4.4.4 Measurements taken during visit 2
- Intraocular pressure
- Best corrected visual acuity
- Ocular surface integrity, including:
    - Conjunctival hyperemia
    - Chemosis
    - Fluorescein staining
- Adverse events

6.4.4.5 Measurements taken during visit 3
- Intraocular pressure
- Best corrected visual acuity
- Ocular surface integrity, including:
    - Conjunctival hyperemia
    - Chemosis
    - Fluorescein staining
- Adverse events

6.4.4.6 Measurements taken during final visit
- Intraocular pressure
- Best corrected visual acuity
- Cup-to-disc ratio
- Visual fields determined by computerized perimetry
- Central corneal thickness, determined by pachymetry

Ocular surface integrity, including:
- Conjunctival hyperemia
- Chemosis
- Fluorescein staining

- Goblet cell density
- Ocular comfort index
- Adverse events

6.4.4.7 Measurements taken for safety call
- Adverse events

6.5 Chronogram and study diagram

Screening test represents a visit that takes place 5 to 28 days before the baseline visit. Such period of time is related to the washout period, which in turns depends on the previously administered hypotensive drug(s).

The **Baseline visit** must be performed strictly after the washout period with a window of 2 days.

Visit 1 is to be performed 14 days after starting the treatment, with a window of ±2 days.

Visit 2, visit 3 and final visit must be performed after 28, 56 and 93 days, respectively, with a window of ±3 days.

Safety call must be performed at day 106, with a window of ±3 days.

Procedure	Visits						
	S	**B**	**1**	**2**	**3**	**Final**	**SC**
	Days						
	-5 a -28	0	14 ± 2	28 ± 3	56 ± 3	91 ± 3	106 ± 3
Eligibility criteria	X	X					
Signed informed consent	X						
Pregnant test (if applicable)	X			X	X	X	
General/ ophthalmic clinical history	X						
Instruction of starting the washout period	X						
Subject code assignment		X					
Treatment group assignment		X					
Drug supply		X		X	X		
Drug return				X	X	X	
Adherence assessment			X	X	X	X	
Tonometry	X	X	X	X	X	X	
Best corrected visual acuity	X	X	X	X	X	X	
Cup-to-disc ratio	X	X				X	
Computerized perimetry		X				X	
Pachymetry		X				X	
Surface integrity evaluation	X	X	X	X	X	X	

Procedure	Visits						
	S	B	1	2	3	Final	SC
	Days						
	-5 a -28	0	14 ± 2	28 ± 3	56 ± 3	91 ± 3	106 ± 3
Impression cytology		X				X	
Ocular comfort index		X				X	
Adverse event assessment			X	X	X	X	X
Concomitant drugs assessment	X	X	X	X	X	X	X
Assessment for subject stay			X	X	X		
Diary handed over to subjects		X	X	X	X		
Subject diary review			X	X	X	X	
Safety call							X

S = screening, B = baseline, SC = safety call

Table 11. Study Chronogram

6.5.2 Procedures to follow in each visit

The following are the procedures to be followed in each visit, as well as a brief description of each one of them.

6.5.2.1 Screening visit

- Eligibility criteria: the PI must include subjects in the study if they meet all inclusion criteria and do not meet any exclusion criteria. See 6.2 Eligibility criteria.
- Signed informed consent: subjects must sign a written informed consent. See 10.3 Consent.
- Pregnancy test: it refers to a quick pregnancy test performed in all women with an age of pregnancy potential who would like to be enrolled in the study. Fertile age is understood as an age at which women have not presented menopause, defined as 12 months since the last menstruation in women with more than 40 years old or women who have undergone hysterectomy or bilateral oophorectomy. Fertile age women using birth control methods, including bilateral tubal obstruction, are subject to pregnancy tests too. Pregnancy tests must be performed by the PI or a team designee by following the instructions of the device supplied by the sponsor.
- General/ ophthalmic clinical history: it refers to any technical, clinical and legal document in which the patient health conditions, medical actions and any other procedures performed in the patient are chronologically recorded. It includes anamnesis and full ophthalmological examinations that help to determine the patient eligibility. If any patient comes from the visit established by the study center, his/her existing clinical history can be used for the purposes of the study, provided the corresponding updates be made.
- Instruction of starting the washout period: it means the suspension of hypotensive drug administration, as per Table 9 Washout period.
- Tonometry: see 6.4.3.8 Intraocular pressure.
- Best corrected visual acuity: see 6.4.3.2 Best corrected visual acuity
- Cup-to disc ratio: see 6.4.3.9 Cup-to-disc ratio

- Surface integrity evaluation: see 6.4.3.5 Ocular surface integrity.
- Concomitant drugs assessment: the PI must query about the drugs the patient used before, during and after the study. All active substances, doses and periods of time must be recorded in the corresponding section of the CRF by the PI.

6.5.2.2 Baseline visit

- Eligibility criteria: see 6.5.2.1 Screening visit
- Subject code assignment: it means the assignment of numbers that will identify the patient throughout the whole study. The assignment must be performed as per section 6.2.4, Subject identification.
- Treatment group assignment: it means the determination of the treatment the patient will receive throughout the study. It must be performed as per Section 7, Methods. Intervention assignment. This assignment is to be performed at the baseline visit (day 0) and must take place simultaneously with the instruction to start the treatment period the next day (day 1).
- Drug supply: the investigation center must supply study patients with the test drug. It must be performed as per sections 6.3.1. Administered treatments and 6.3.4.1. Supply and reception. After each drug supply, a training/retraining on the study drug application must be performed.
- Best corrected visual acuity: see 6.4.3.2 Best corrected visual acuity
- Tonometry: see 6.4.3.8 Intraocular pressure.
- Cup-to-disc ratio: see 6.4.3.9 Cup-to-disc ratio.
- Computerized perimetry: see 6.4.3.1 Visual fields. If the test subject has undergone previous perimetry and met the established requirements, the results of such perimetry might be used for the purposes of the trial provided the period between the date of the previous taste and the inclusion date is no greater than 6 months.
- Pachymetry: see 6.4.3.6 Corneal pachymetry.
- Surface integrity evaluation: see 6.4.3.5 Ocular surface integrity.
- Impression cytology: see 6.4.3.3 Goblet cell density.
- Ocular comfort index: see 6.4.3.4 Ocular comfort index.
- Subject diary submission: the PI must hand over the instrument “subject diary” to the subject.
- Concomitant drugs assessment: see 6.3.3 Allowed and prohibited concomitant treatments and interventions before and after the study.

6.5.2.3 Visit 1

- Tonometry: see 6.4.3.8 Pressure.
- Best corrected visual acuity: see 6.4.3.2 Best corrected visual
- Surface integrity assessment: see 6.4.3.5
- Subject diary submission: see 6.5.2.2 visit.
- Subject diary review: the activity from the PI in which the diary tool is collected from the subject and its correct completion is evaluated.
- Adherence assessment: see 6.3.2
- Adverse events assessment: see 6.4.3.7
- Concomitant drugs assessment: see 6.3.3
- Continuity assessment: the determination from the PI and the subject’s desire to continue with is participation in the study.

6.5.2.4 Visit 2 and 3

- Pregnancy test: see 6.5.2.1 visit.
- Drug return: the delivery of the drug from the research subject to the center of the study. See 6.3.4.1 Returns.
- Drug delivery: the delivery of the drug from the patient in the study, from the research center. This will be performed according to section 6.3.4.1 and reception.
- Adherence evaluation: see 6.3.2
- Tonometry: see 6.4.3.8
- Best corrected visual acuity: see 6.4.3.2 Best corrected visual capacity.
- Surface integrity assessment: see 6.4.3.5
- Subject diary review: see 6.5.2.3 Visit 1.
- Subject diary submission: see 6.5.2.2 visit.
- Adverse events evaluation: see 6.4.3.7 events presence.
- Concomitant drugs assessment: see 6.3.3 and forbidden concomitant treatments and interventions before and after the study.
- Continuity assessment: see 6.5.2.3 Visit 1.

6.5.2.5 Final visit

- Pregnancy test: see 6.5.2.1 visit.
- Drug return: the delivery of the drug from the research subject to the center of the study. See 6.3.4.1 Returns.
- Adherence assessment: see 6.3.2
- Tonometry: see 6.4.3.8 pressure.
- Best corrected visual acuity: see 6.4.3.2 Best corrected visual capacity.
- Cup/disc ratio: see 6.4.3.9 ratio.
- Computerized perimetry: see 6.4.3.1 fields. In final computerized perimetry, a window period of ± 5 days will be allowed.
- Pachymetry: see 6.4.3.6 pachymetry.
- Surface integrity assessment: see 6.4.3.5
- Impression cytology: see 6.4.3.3 cells density.
- Ocular comfort index: see 6.4.3.4 comfort index.
- Subject diary review: see 6.5.2.3 Visit 1.
- Adverse events assessment: see 6.4.3.7 events presence.
- Concomitant drugs assessment: see 6.3.3 and forbidden concomitant treatments and interventions before and after the study.

6.5.2.6 Safety call:

- Adverse events assessment: see 6.4.3.7 events presence.
- Concomitant drugs assessment: see 6.3.3 and forbidden concomitant treatments and interventions before and after the study. The antihypertensive agent with which the subject study continued the trial will be recorded.

6.5.3 Study diagram

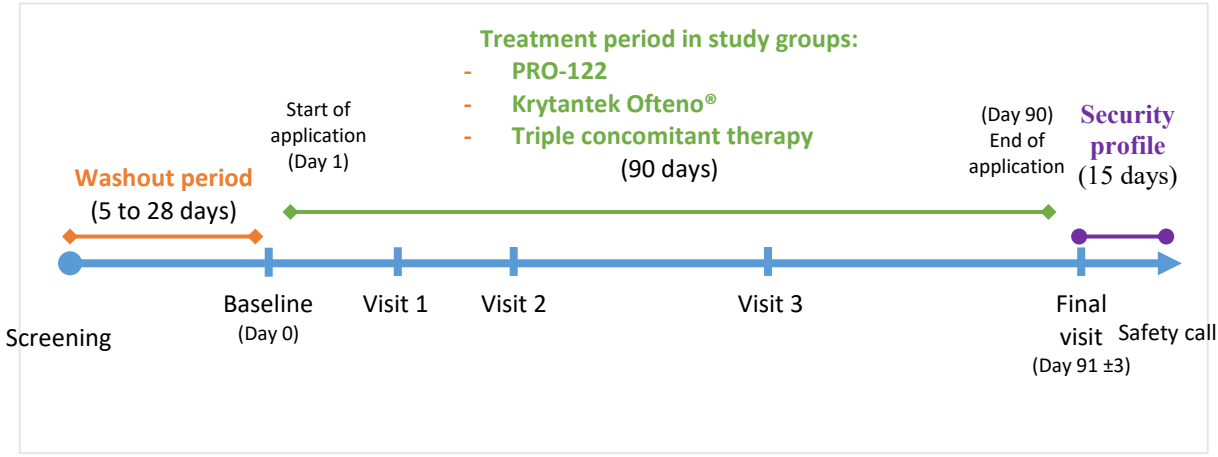


Figure 4. Study diagram

6.6 Sample size

The simple size calculated for this study is 51 subjects.
Each group will consist of 17 subjects and there will be three intervention groups.

6.6.1 Sample size calculation

Sample size was calculated using the formula for continuous quantitative variable in clinical trials, [9] the data were also entered into the online calculator, software developed by David Schoenfeld, Ph.D. with support by the MGH Mallinckrodt General Clinical Research Center. [63]

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

With a 95% statistical confidence, corresponding to type I error, equal to 1.96, with an 80% potency, corresponding to type II error, equal to 0.84. A standard deviation was considered from the intraocular pressure value measured on day 90, after the use of a 2.0 mmHg triple fixed combination (timolol + brimonidine + dorzolamide). The calculated expected difference in comparison with the intraocular pressure of the double 2.2 mmHg fixed combination group (timolol, brimonidine), reported in a clinical trial for the assessment of efficacy and safety with 212 Mexican subjects with primary open-angle glaucoma or intraocular hypertension. [31]

According to the previous calculation, the result is 13 subjects (12.5) per group. The total amount when considering 3 intervention groups is 39 subjects, which was incremented by a 20% (8 subjects) by the possible losses. The total size of a required sample is 47 subjects. This is the reason why each group will consist of 17 subjects.

Per group n = 17 subjects, one ye will be studied in the efficacy analysis.

Total n = 51 subjects, one eye will be studied in the efficacy analysis.

Considering an analogue method developed by *Gandolfi S, et al.* [64] in which the reduction of the intraocular pressure modification with the fixed antihypertensive therapy comprised by brinzolamide 1%/brimonidine 0.2% is compared against the concomitant brinzolamide 1% + brimonidine 0.2%

The sample size was calculated using the formula for continuous quantitative variables in clinical trials.

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

With a 95% statistical confidence corresponding to type I error, equal to 1.96, with an 80% potency, corresponding to type II error, equal to 0.84. A standard deviation was considered from the baseline difference value – final of the intraocular pressure measured at 3 months at 9:00 hours in 373 patients with primary open-angle glaucoma or intraocular hypertension, after the use of the 0.16 mmHg dual concomitant therapy (brimonidine + dorzolamide). [64] The difference in the IOP expected in comparison with the patients who received antihypertensive therapy in a double fixed combination (brinzolamide/brimonidine) would be at least 0.2 mmHg.

According to the previous calculation, the result is 12.0 per group. The total amount when considering 3 groups is 36 subjects, which was incremented by a 20% (6 subjects) by the possible losses. The total size of the required sample is 42 subjects. This is the reason why each group will consist of 14 subjects.

Per group n = 14 subjects, one eye will be studied in the efficacy analysis.

Total n = 42 subjects, one eye will be studied in the analysis.

Since the sample size calculation, using the values stated by *Baiza L, et al*., it was decided that a total of **51 patients** with primary open-angle glaucoma or ocular hypertension was evaluated on this research protocol.

6.7 Recruitment

It is recommended that, during the development of this research protocol, the principal investigator requests the approval of the Research Ethics Committee and the Research Committee, as well as the authorization of the corresponding regulatory entity, to publish or disseminate in mass media, an invitation to participate in the trial for people with an incorrect antihypertensive control.

It is possible to comment with other healthcare professionals, especially with those evaluating patients with chronic and degenerative diseases about the opportunity for them to receive gratuitous treatments, appropriate ophthalmologic assessment at no charge, as well as clinical studies which will allow to determine their ocular clinical status in a more precise way by participating in a clinical research protocol sponsored by Laboratorios Sophia, S.A. de C.V.

7. Methods. Intervention assignment

7.1 Assignment sequence generation

The random numbers will be generated by the online tool: www.randomization.com

3 strata corresponding to the intervention groups will be used, said strata will be balanced for a research center. Assignment will be 1:1:1.

7.2 Blinding mechanism

Blinding will be performed by the personnel assigned by the Laboratorios Sophia S.A. de C.V. Clinical Operations Management. The blinding will consist in the elimination of the primary label (commercial) for Krytantek Ofteno® and the triple concomitant therapy and the placement of a label identical to those of the other interventions. Since the bottles in which Krytantek Ofteno® and the concomitant therapies are different in color and cap shape to the ones used for the placebo and the PRO-122, a masking will be performed in the primary packaging, which shall be identical for all three interventions.

7.3 Implementation

The assignment sequence will be generated by the Laboratorios Sophia S.A. de C.V. Clinical Operations Management. The research center will receive a set of envelopes which will have an individual intervention number. The envelopes will be identical on the outside. Each one of said envelopes will be shown to the participants for the principal investigator or a designated member of the investigator's team to choose.

7.4 Blinding (Masking)

Blinding will be for the research subject and the principal investigator. Also, the statistical analysis will be performed in a blind manner in case a partial and final analysis is performed.

Masking will be performed using identical boxes in primary packaging in all three groups. Blinding for the research subject and the investigator will consist in the replacement of the commercial labels for the comparator in the bottles and the use of identical labels containing an assignment number.

7.4.1 Blinding opening

Blinding might be opened in the following cases:

1. Presence of a serious adverse event.
2. Security alarm by the use of study drugs.
3. In case the sponsor considers so by any safety reason or any other relevant motive.

8. Methods. Collection, administration and data analysis.

8.1 Data recollection methods

A clinical monitor will be assigned to each research center, said monitor will be authorized to watch, review, procure and ensure the quality of the information obtained from the participants is trustworthy and reliable. Each monitor will schedule periodic visits to the research centers in order to review the source documents and verify all the information captured in the CRF. All clinical monitors will be trained regarding the study protocol information (objective, visits, procedures, accepted values range, etc.) In case the data are not identical between both records, the clinical monitor will generate a discrepancy, which will be resolved by the research center in the time the sponsor deems reasonable to fulfill the objectives of the clinical trial. The correction of discrepancies will be performed according to Good Documentation Practices.

The data recorded in the CRF will be reviewed by Laboratorios Sophia personnel, trained in the ophthalmologic, clinical and pharmaceutical areas; the personnel will have the ability to generate discrepancies in case the data do not adhere to the stipulations in the research protocol or put the participants in risk.

Once all discrepancies generated by the clinical monitors team and the clinical personnel are resolved, data will be downloaded in an electronical database (Excel worksheet) by personnel designated by the sponsor. A new review of the data will be made to verify the reliability of said data and new discrepancies can be generated in case it is considered to do so.

The generated database will be safeguarded by the sponsor and only the designated personnel will have access to it.

8.1.1 Follow-up completion strategies

- Participants will be given clear information on the importance of the study and the benefits the population will get from its results.
- Participants will be given aid for transport in order for them to attend their visits.
- Calls or messages will be made or a printed calendar will be given in order to remind the participants about their appointments and the activities to be performed and their estimated duration time.
- In case the participant subject does not attend the appointment, the research center shall make a phone call to know the reason and will try to arrange a new appointment in the established window period or an non-scheduled appointment
- In case the arrangement of an appointment is not possible, the participant will be questioned about the presence of adverse events and the motive of the study withdrawal as minimum data.

8.2 Data administration

The subject medical record (including clinical notes, test results, etc.) as well as the subject's diary and the OCI questionnaire are considered as source data.

The PI or the designated person of its team will fill the Case Report Form (CRF) as well as all the other documents provided by the sponsor (for example, the documents related with the treatment management).

An electronical CRF was designed to record the data required in the protocol and that the investigator gathers in each one of the visits.

For the self-assessment questionnaires it is not allowed that the principal investigator or the person in charge of filling modifies the parts written by the trial subject.

The data recollection in the investigator's site will be performed by the investigator him/herself or a designated person of its team after elaborating the Medical Record. The investigator or a designated person of its team will be trained regarding the filling of the CRF.

All the corrections of the CRF data shall be made by the investigator or the designated person of its team according to the provided instructions.

To ensure the confidentiality and safety of the data, user names and access codes will be used to restrict access to the system only to authorized personnel.

The monitor shall ensure all the fields of the CRF are filled. After comparing the data against the source documents, the monitor will request the investigator to make a correction /clarification to be responded and closed as soon as possible.

The Laboratorios Sophia S.A. de C.V. Scientific Committee will provide a last medical-scientific review and set the tone to lock the database.

8.3 Statistical methodology

8.3.1 Primary and secondary outcome variables

The statistical analysis will be performed by the Laboratorios Sophia. The version 19 SPSS statistical program will be used.

The designated personnel will be blinded to the intervention groups. Codification will be performed using consecutive numbers for each intervention group.

Data will be recollected and arranged in an Excel program worksheet. After this, data will be exported to the SPSS program platform. Variables will be categorized according to their nature.

The result of the continuous quantitative variables will be presented as measures of central tendency: mean, standard deviation and ranges. See **¡Error! No se encuentra el origen de la referencia. measurement method.** The total sample size will consider an eye as a case for the assessment of efficacy. In patients whose both eyes are eligible for the study, the right eye will be studied for the analysis.

The normal results distribution will be obtained by the Kolmogorov-Smirnov and Shapiro-Wilks tests, as applicable.

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

- Intra-group analysis: Wilcoxon range test.
- Inter-group analysis: Mann-Whitney U test.

For the analysis, IOP measurements before the application of the intervention and two hours after will be considered. The difference level to consider significance will be 0.05 alpha or less.

Nominal and ordinal qualitative variables result will be presented as frequencies, ratios and percentages. See **¡Error! No se encuentra el origen de la referencia.**

Statistical analysis to identify significant differences of the qualitative variables will be performed creating 2 x 2 contingency tables and it will be performed as follows:

- Intra-group difference: McNemar test.
- Inter-group difference: Pearson's $\chi 2$ (chi square) test or Fisher's exact test in expected values less than 5.

The difference level to consider significance will be 0.05 alpha or less.

For the report of adverse events, all eyes of participants randomly assigned to an intervention group will be considered. Results will be expressed in a number of cases and both eyes will be considered.

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

8.3.2 Additional analysis

An internal analysis will be performed to know the changes occurring between each visit. Also, a comparison between the IOP previous to the application of any of the interventions and the change 2 hours later; however, these results will not be considered as part of the efficacy analysis.

8.3.3 Population analysis and missing data management

The efficacy analysis will considered those cases in which the measurements of the baseline and final visits are completed. Those subjects without some of these measurements shall not be integrated to the final database to assess efficacy. In case the efficacy variables measurement

cannot be performed in the right eye, due to medical reasons or other, the left eye data will be used.

The safety assessment will include in the analysis all those subjects (considering one eye or both) who have been exposed at least once to any of the interventions regardless of the visit in which they were eliminated from the study.

9. Methods. Monitoring

9.1 Monitoreo de datos

The monitoring visits from a site monitor from Laboratorios Sophia, S.A. de C.V. have the purpose of confirming the studies sponsored by Laboratorios Sophia, S.A. de C.V. are performed according to the ethical principles from the Declaration of Helsinki which are consistent with the Good Clinical Practices and with the applicable regulatory requirements (verifying the continuous protocol compliance, amendment (s), reviewing the accounting logs of the research product, verifying the site personnel and the facilities remain appropriate to perform the study).The investigator shall assure to have enough time, space and qualified personnel for the monitoring visits.

In order to perform the monitoring review, it is obligatory to provide a direct Access to all source data and data regarding the study site. The monitor will perform a review of the CRF and a Source Document Verification (SDV). An SDV is the verification of the CRF records via a comparison with the source data provided by the investigator for this purpose.

Regarding the CRF, the monitor will mark the complete and approved screens in every visit.

According to applicable regulations, the Good Clinical Practices and the Laboratorios Sophia, S.A. de C.V. procedures; Laboratorios Sophia, S.A. de C.V. monitors will contact the site before the start of the trial to review, along with the site personnel, the protocol, the regulatory, ethical and Laboratorios Sophia, S.A. de C.V. requirements. When reviewing the data collection procedures, the discussion will also include the identification, agreement and documentation of individual data for which the individual CRF records function as source document.

Laboratorios Sophia, S.A. de C.V. will monitor the study to verify, among other things, the following:

- The data are authentic, correct and complete.
- The safety and rights of the subjects are being protected.
- The study is being performed according to the currently approved protocol, any other study agreement, Good Clinical Practices and all applicable regulatory requirements.

The investigator and Head of the medical institution (whenever applicable) agree to allow the monitor to have direct access to all relevant documents.

The study monitoring visits will be performed at regular intervals, depending on the recruitment rate, according to the arrangements made by the investigator and the sponsor. All information related to these visits will be handled as strictly confidential.

When completing or prematurely discontinue the study, the monitor will perform the activities for the site closure along with the investigator or the site personnel, as appropriate, according to the applicable regulations, Good Clinical Practices and Laboratorios Sophia, S.A. de C.V. procedures.

After the study is closed, the investigator shall keep all the records from the study in the site in a safe place. All records shall be kept to allow for their easy and appropriate retrieval, whenever necessary (for example, in an audit or an inspection). Laboratorios Sophia, S.A. de C.V. will inform the investigator/institution about the time period in which these records shall be kept in order to comply with all applicable regulatory requirements; however, the investigator/institution shall seek the written approval of the sponsor before proceeding to the elimination of these records. The minimum retention time will satisfy the strictest applicable standard to that site for the study, according to the GCP dispositions, any institutional requirement or the applicable laws and regulations or the Laboratorios Sophia, S.A. de C.V. standards/procedures.

The investigator/institution shall notify Laboratorios Sophia, S.A. de C.V. about any change in the file arrangement including, but not limited to, the following: file in an off-site facility, property transfer of the records in case the investigator leaves the site.

9.2 Preliminary analysis and early study termination

The partial analysis will allow the sponsor to take a decision on the early termination of the study in case the safety of the participants is compromised.

The early termination study will be considered in the following cases:

1. Presence of serious adverse events in more than 5% of the participants in each intervention group.
2. The competent authority (e.g. COFEPRIS) considers termination due to safety alerts.
3. The sponsor determines termination due to self-interest or events such as: financial support, manufacturing errors, etc.
4. Recruitment lower than expected.

In case the decision is early clinical trial termination, this will be informed within the first 24 hours to all research centers, via the available communication paths. Also, this will be informed to the corresponding authority in each country (if applicable) and to the involved Ethics Committees.

Each research center has an obligation to inform the subjects participating in the clinical trial in a period no longer than 24 hours, after receiving the information from the sponsor. All involved subjects in any phase of the study shall be informed.

The Laboratorios Sophia, S.A. de C.V. Clinical Operation Management and Medical Direction will be in charge of the preliminary assessment and will have the ability to determine the fate of this protocol, as they deem appropriate.

9.3 Adverse events

9.3.1 Investigator responsibilities

To perform the adverse events verification via interrogation, relevant physical examination, and assessment of progress as well as the appropriate medical and pharmaceutical management, resolution or outcome and definitive discharge following the definitions determined in the national and international regulations. [65] [66] [67]

In case of adverse events or any occurrence which compromises the health and wellbeing of the patients, relevant medical services will be provided in the research site or the patient will be referred to the hospital center with the best health care capabilities with which the research site or investigator has a medical attention agreement. The investigator shall notify the sponsor’s clinical monitor, according to the periods established in the national and international regulations. In case of serious

adverse events, the sponsor shall be notified and the corresponding information shall be recorded in the case report form and the Research Ethics Committee and the Research Committee shall be informed also.

The treatment of adverse events shall be performed according to the event attention diagram (See **Figure 5. Attention to the adverse** event).

In the final report elaborated by the Laboratorios Sophia, S.A. de C.V. Clinical Operations Department Scientific Committee, the adverse events report shall be included in compliance of the current national and international regulations. [65] [66]

9.3.1.1 Adverse events record in the Case Report Form

The record of adverse events considers the information regarding the identification data of the participating patient such as code, age, sex, left eye, right eye.

Information about the type of adverse event, adverse reaction or investigational drug or product adverse reaction suspicion, as appropriate. The date on which the adverse event is occurred as well as the date in which the investigator is notified about it, outcome or resolution date, as applicable. Clinical diagnosis is indicated. If there is a lack of therapeutic response to the investigational drug or product, this shall be notified as an adverse reaction. In concomitant drugs, the used therapy for the pharmacological management of the adverse event, adverse reaction suspicion or adverse reaction shall be included. Record the outcome or resolution of the event: patient recovered without side effects, patient did not recover. Patient died due to the adverse reaction/event, patient died and it is considered that the drug might have contributed to this, patient died and death is not related to the investigational drug/product or also state that the event consequence is unknown.

Record information on the investigational drug or product or the drug associated with the adverse event, adverse reaction or adverse reaction suspicion. As applicable, the information regarding the generic name, nonproprietary name or code of the investigational drug or product, according to the methodological design of the study; this is relevant in the case of blinded studies or studies in which placebos are used as comparators, since there are circumstances that justify the opening of the blinding procedure to determine if the adverse event, adverse reaction or adverse reaction suspicion might be attributable to the active agents or to the pharmacologically inert substance (s), as might be the case of vehicles or additives, according to the clinical investigation phase in which the drug development is in.

It will also be necessary to capture the data relating to the a) lot number, b) manufacturer laboratory, c) expiration date, d) dose, e) route of administration, f) dates of start and g) term of the Administration and/or consumption, because of the prescription. According to whether it's an investigational product or medicinal product (a protocol currently involving the patient), or either it is a medication consumed by the subject in research as a base for the treatment of comorbid diseases or that it is used for the handling of any sign or transient symptoms that does not correspond to the Natural history of pathology that motivated his entry into the research protocol.

Record the removal or maintenance of the drug, investigational product or medicinal product, as appropriate. Indicate if at the moment of the withdrawal of the investigational product or medicinal product or the suspicious (of the event) drug product, the adverse event disappears. Also indicate if a dose adjustment is made, if the event changes in terms of intensity or severity and persistence of the reaction. It is important to indicate if the adverse reaction or event reappears in those patients that are exposed again to the drug or investigational product or drug product, which previously had been suspended.

Regarding the concomitant pharmacotherapy. Indicate the generic name, dose, route of administration, dates of start and term of its employment, as well as the reason of its prescription regardless if it is according to the prescribing information or data sheet, or if it is employed out of the norm or of what has been authorized by the local, national or international regulatory entity.

Concerning the relevant medical history. The analysis of the adverse event, adverse reaction or suspected adverse reaction considers the information previously narrated, however the clinical context in which the said damaging phenomenon in the participants of the Clinical research Protocol is of special interest. Consequently, if the investigator considers convenient to mention the information about the participant's previous condition, hypersensitivity or allergy phenomena, previous surgical procedures, laboratory or cabinet tests which the participant has undergone, etc., he/she has to do so. In case of having enough space in the case report format, the information can be completed in his/her clinical note, on the medical record.

9.3.1.2 Monitoring of adverse events

The PI will provide care and conduct the AE that the participant shows until the outcome of it, according to what it is refer into the following paragraph.

9.3.1.3 Procedures for a serious adverse event

The process of care of the adverse event considers the following stages:

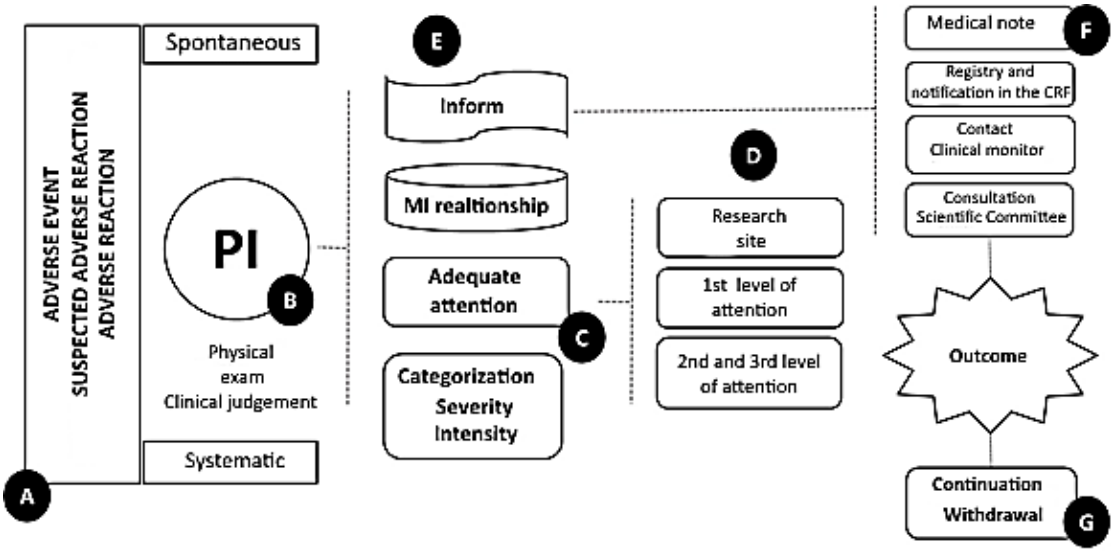


Figure 5. *Attention to the adverse event*

A. During the development and conduction of this clinical research the patient can present undesirable adverse events or adverse reactions, of medical involvement, which do not necessarily have a causal relationship with the investigational product or drug product. These damaging phenomena can occur through the usage of the investigational drugs, in an unintended way, with licensed doses for human use granted by a local, national or international regulatory entity, whether for the prophylaxis, diagnosis, treatment or modification of any physiological process. However it may be suspected that the investigational drug or product or the placebo cause some unwanted clinical manifestation. The adverse events, adverse reactions or suspected adverse reactions to one or more drugs

can occur during the systematic evaluation of the participants (in the days when the clinical review, according to the schedule of activities, is scheduled) or suddenly , in such a way that,

B. The investigator must be the first person to who the patient notifies that he/she has developed or presented any damaging phenomenon of clinical nature during his/her participation in the present Protocol of research.
C. According to his/her clinical trial, based on the relevant physical exam, interrogation, etc.; as well as to the analysis of the information available in the medical literature and of the referred in investigator manual; the prescribing information or data sheet of the comparator drug, the principal investigator determines the relevant attention of the damaging event/ reaction; either:
D. in the site of research or in the hospital of greater resolution capacity (1st, 2nd or 3rd level of medical care). In such a way that, if the investigator sends the patient to the hospital, he/she participates as a reference system. It can be through an ID card which indicates that the patient belongs to this research and there is a number of trade or folio, which belongs to the Convention of urgent care with the health institution of greater resolution capacity or by means of a medical reference note issued by the principal investigator so that the proper attention is provided to the participant patient. It should be noted that the sponsor of the study, Laboratorios Sophia, S.A. de C.V., will pay the expenses for the participant patient medical care, only if the adverse event, adverse reaction or suspected adverse reaction to a drug is related or associated with the investigational drug product or product.
E. Taking the clinical information collected, whether during the attention on the research site or provided by the treating physician(s) at the hospital, the principal investigator records the adverse event, suspected adverse reaction or adverse reaction to medicine in his/her clinical note of the clinical file recording its seriousness, intensity (mild, moderate or severe), its relation to the investigational drug or product , as well as
F. The migration of the relevant data to the case report format and to its respective adverse event section. Indicate the relevant information, already referred to in section 9.3.1.1., by virtue of that in the cases of serious adverse events, which should be reported in less than 24 hours after the time in which the principal investigator have knowledge thereof, the clinical study monitor is aware, in order to inform to the Scientific Committee and the sponsor's Pharmacovigilance department and subsequently to inform to the Research Ethics Committee. Regarding to the non-serious adverse events, these will be registered and properly cared, and in the final report of the clinical trial the regulatory entity must be informed about the safety profile of the investigational product or drug product.

Registration of the outcome of the adverse event, suspected adverse reaction or adverse reaction depends substantially on the monitoring that the principal investigator made to the participant, as it is expected that most damaging phenomena, see section of the safety profile in paragraph 5.3 and in the investigator manual, are of ophthalmic nature, there may however be systemic alterations. Therefore, according to the investigator, the patient withdrawal or permanence will be considered in accordance with the provisions taken in section 6.2.3 Elimination Criteria of this Research Protocol.

9.3.1.4 Causality assessment

The causality assessment of the methodology used to estimate the probability of attributing the adverse reaction, the suspicious of the same or the observed adverse event to a drug, investigational product or drug product, considers probabilistic categories according to the available evidence and to the information quality, based on the national pharmacovigilance standards.[65] As a tool to facilitate the causality probabilistic categorization, the principal investigator can use the Karch and Lasagna's algorithm modified by Naranjo referred by Aramendi I, 2011 in which different items are

graded which allow to assign a value to the cause and effect relationship between the drug administration and the adverse reaction.[68] See table **¡Error! No se encuentra el origen de la referencia.**

Karch and Lasagna's algorithm modified by Naranjo			
No.	**Reagent**	**Score**	
		Yes	**No**
1.	There are previous conclusive reports on adverse reactions to medication, adverse event or suspected adverse reaction to medication.	+1	0
2.	The adverse event occurred when it the suspicious medication was administered	+2	-1
3.	The adverse reaction to medication, adverse event or suspected adverse reaction to a medication improved upon suspension or with the administration of a specific antagonist	+1	0
4.	The adverse reaction to medication/ adverse event/ suspected adverse reaction to medication appeared again with the administration of the drug/ investigational product/drug product	+2	-1
5.	There are alternative causes which can cause this reaction	-1	+2
6.	The adverse reaction/ event adverse / suspected adverse reaction to medication occurred after the placebo administration	-1	+1
7.	It was determined the concentration of the drug into the bloodstream or other liquids in toxic concentrations	+1	0
8.	The intensity of the adverse reaction/ adverse event / suspected adverse reaction to medication was greater at higher doses or less with lower doses	+1	0
9.	In the past, the patient has had similar reactions to the investigational product/drug product.	+1	0
10.	The adverse reactions/ adverse event/ suspected adverse reaction to medication was confirmed by any objective evidence	+1	0
	Total score	sum	
Probabilistic category based on the score obtained			
I	The causal relationship is verified	≥,9	
II	It is possible that the RAM is due to the medication or the investigational product	5 to 8	
III	It is possible that the RAM is due to the medication or the investigational product	1 to 4	
IV	The causal relationship is questionable	0	

Shows the reagents considered by the Karch and Lasagna's algorithm modified by Naranjo where everyone receives a defined score and the final sum allows to estimate the probabilistic category of the cause-effect relationship between the administration of the investigational product/ drug product, and the adverse reaction, adverse event or suspected adverse reaction. Consider that if the information is not available, a score equal to zero should be registered.

Table 12. Karch and Lasagna's algorithm modified by Naranjo

So that the degree of certainty to establish as an agent of the damaging phenomenon occurring to the participant patient to the investigational product or investigational drug product (as appropriate), can be directly indicated by the principal investigator on the basis of his/her clinical experience or by the voluntary application of the aforementioned tool. However, it is important that the investigator

takes into account the following arguments in favor of the causal relationship: the strength of association refered to the number of cases related to the exposed. The consistency of the data, i.e. the presence of a feature or common pattern. The exposition-effect pattern: that determines the relationship with the site of manifestation, time, dose and reversibility after discontinuation. Biological plausibility: referring to the possible pharmacological or pathophysiologic mechanisms involved in the development or presentation of the adverse event. Experimental findings: for example the appearance of abnormal metabolites or high levels of the biotransformation of the drug product or product. Analogy: the acquired experience with other related drugs, adverse reactions often produced by the same family of pharmacological agents. Nature and characteristics of the data: objectivity, accuracy and validity of the relevant documentation.[69]

9.3.2 Responsabilities of the sponsor

The sponsor will be responsible for, and will cover the medical care costs of the adverse events related to the investigational product.

9.4 Audit

To ensure compliance with all applicable regulatory requirements and the GCP, Laboratorios Sophia S.A. de C.V. could carry out a quality assurance audit. The regulatory agencies could also carry out a regulatory inspection to this study.

9.4.1 Study pre-audit

The study centers included in the study will be subject to a feasibility visit prior to the selection of the Center, which will verify that the facility complies with the minimum requirements indicated by the sponsor.

9.4.2 Audit/inspection during the conduct of the trial

May take place at any time: prior, during or after the conclusion of the study. If any audit or inspection is performed, the investigator and the institution must agree to allow the auditor/inspector to have direct access to all relevant documents, and will assign their time and that of its staff to the auditor/inspector in order to discuss the findings and any outstanding issue.

10. Ethical considerations

10.1 Approval of the commitees

This study will be performed according to the Declaration of Helsinki guidelines, World Medical Association 2013. Nuremberg Code; Nuremberg trial by the International Tribunal at Nuremberg, 1946 Belmont Report, National Commission for Protection of Human Subjects of Biomedical and Behavioral Research, 1979. It will be led in accordance with the scientific and technical requirements needed for the registration of human medicinal products of the Good Clinical Practice guide of the International Council for Harmonization. International Ethical Guidelines for the Biomedical Research in Humans of the Council for International Organizations of Medical Sciences, CIOMS, 2002. International Ethical Guidelines for epidemiological studies of the Council for International Organizations of Medical Sciences, CIOMS, 2008.

The Research Ethics Committee and the Research Committee will evaluate the Protocol before carrying out the study and they will issue its approval or possible modifications for its realization, these committees must be notified of any significant change to the Protocol. Besides the above, the current

regulations issued by the Ministry of Health or their counterpart in the correspondent countries, shall be complied. The Mexican General Health Law (Ley General de Salud), NOM 012 Official Mexican Standard NOM-012-SSA3-2012, that establishes the criteria for the execution of research projects for health in humans. The Argentinian National Administration of Food, Drugs and Medical Technology (ANMAT) 6677/2010 disposition. The study is considered as a research with greater risk than the minimum according to the regulations of the Mexican General Health Law in the Research for health, Title II, Chapter I, Article 17, Category III, published in the Official Gazette on January 6, 1987.

The principal investigators or the studies coordinators or the authorized personnel by the sponsor will submit for evaluation, by the Research Ethics Committees, the Research Committees and when apply by the Biosafety Committee, the investigational project essential documentation: Research protocol, the Informed consent letter, the investigator manual, the subject journal, as well as the ones additionally requested, according to the local , national or international requirements applicable by the regulatory entities.

The study will not start at the research site if the confidentiality agreements and the economic proposal of each of the principal investigators doesn't exist, duly signed and without the previous favorable opinion and/or the approval of the Research Ethics Committees, Research, Research Committees and, when apply, by the appropriate Biosafety Committee.

The study will not start if the relevant local, national or international regulatory requirements are not fulfilled and if the appropriate Sanitary Authorization is not available.

10.2 Amendments to protocol

The amendment process will be relevant where there is a need to make any changes to a document that is part of the project or research protocol, resulting from changes to the methodology framework, replacing the principal investigator or the risks identification in the research subjects. The documents likely to be amended will be: Protocol, the informed consent letter, the investigator manual, documents for the patient, measuring scale, and the activities schedule.

Any amendment must be approved by the sponsor and/or the principal investigator. The document(s) amended, once reviewed and approved by the Research Ethics Committee and the Research Committee or, where applicable, by the Biosafety Committee, (entities that issued the initial favorable opinion for the research conduction) will be sent for its authorization by the relevant regulatory entity.

The amendments which substantially altered the Protocol or conferred it an additional or different risk to the research subjects must be approved by the Committee. Is responsibility of the investigator to take measures in situations that require an immediate action in order to avoid unnecessary damage to the study participants.

The principal investigator is responsible to inform the Research Ethics Committee about any amendment to the Protocol that could eventually affect the rights, safety and well-being of research participants. Likewise, the investigator must make known any situation or new knowledge that shows an increased risk for the participants, term or premature termination of the study, the reasons and the results obtained so far. Additionally, the investigator must inform about the conclusion of the study, at the moment of the completion of the research protocol.

The list of amendments, and where necessary the issuance of the list of errata, will be referred in the final report of the investigation.

10.3 Consent

10.3.1 Obtaining

The Informed consent must be obtained before the performance of any procedure indicated in the Protocol in the subject.

The written informed consent documents will incorporate the elements of it described in the Declaration of Helsinki guidelines and the ICH guidelines for the Good Clinical Practices and these must be consistent with all the applicable Laws and Regulations.

The PI will provide to the potential participants all the information related to the characteristics of the study, as well as the potential benefits, risks, objectives and procedures of the study.

This information shall be in an understandable language for the subject, it will be explain to the subject who has the right to end his/her participation in the study at any stage, without affecting the relationship with the investigator and/or its future assistance. The Informed consent will be submitted for consideration of the potential participant; he/she must have enough time to analyze each and every one of the aforementioned aspects and in case he/she has a doubt, this will be clarified by the person in charge of obtaining the informed consent. Once the participant accepts to participate in the study, he/she must sign and date the letter of informed consent in the presence of two witnesses who are related or unrelated to the subject of study, they will participate in the informed consent process and sign endorsing that the process was carried out prior to any study procedures, that the study information was clearly explained and that all the doubts were clarified, if any.

In case of a subject is illiterate, the acceptance will be done with his/her fingerprint, and if the subject is not able to give and appropriate informed written consent, a representative of the subject “legally authorized" can provide such consent instead of the subject in accordance with the applicable laws and regulations.

The PI must also sign and date this consent.

The informed consent must be signed in duplicate by all involved, and two witnesses, a copy will be filed in the folder of the investigator and the other will be delivered to the participant. The IP shall document in the clinical history of the subject, the date on which he/she signed the informed consent.

10.3.2 Special considerations

Auxiliary studies to be carried out during the conduct of the trial (pachymetry, perimetry and impression cytology) do not have an invasive nature and pose no additional risk that must be considered part of the listed procedures of the informed consent.

10.3.3 Modification to the informed consent

Any change to the "informed consent" is an amendment to this document and shall be submitted before the Research Ethics Committees, and if applicable, before the competent authorities for its approval.

The amendment shall include a copy of the new version in the language or languages of the country.

Such amendments may be implemented only after obtaining the written approval of the Research Ethics Committe and the Regulatory agency (as applicable), except a required amendment to remove an immediate hazard to the subjects of the study.

Each subject affected by the amendment should fill it, date it and sign two original documents of the new version. A signed original document of the amendment shall be given to the subject and the second original of this document shall be kept by the investigator.

10.4 Confidentiality

All the documents and information provided to the investigator by the sponsor are strictly confidential. The investigator expressly agrees that the data about his/her professional and clinical experience, provided to the sponsor on paper and stored in electronic or digital form, are solely for the related usage to his/her activities with the clinical studies sponsor, in accordance with Good Clinical Practices. The investigator accepts that he/she as well as the members of his/her team will use the information only within the framework of this study in order to carry out the Protocol. This Convention is mandatory as long as the confidential information has not been disclosed to the public by the sponsor. The Protocol of the clinical study, provided to the investigator, may be used by his/herself and by his/her colleagues to obtain the informed consent of the subjects of the study. The Protocol of the clinical study, as any information taken of the same must not be reveal to other parts without the written authorization of the sponsor.

The investigator will not disclose any information without the prior written consent of Laboratorios Sophia, S.A. de C.V., except to the representatives of the competent authorities, and only for their request. In the last instance, the investigator is obliged to inform Laboratorios Sophia, S.A. de C.V. before disclosing the information to these authorities. The investigator will fill and keep a record of the subjects selection as well as the identification and enrollment list of each of the participant subjects in the study. The investigator agrees to give access on the site to the auditor or the representatives of the competent authorities. The information will be treated pursuant to professional secrecy.

10.5 Declaration of interests

The PI is committed to make a declaration of financial interests and conflict of interests prior to the start of the study.

10.6 Access to information

The final study database will be property of Laboratorios Sophia, S.A. de C.V. and its access will be restricted. The PI won't have access to this, except with the prior written permission of the Sponsor.

10.7 Auxiliary and post-study-termination cares

The care of the AE will be carried out according to the section 9.2 Adverse Events.

10.8 Biosafety aspects

WITHOUT IMPLICATIONS FOR BIOSAFETY

This protocol titled: "Phase III clinical study, to evaluate the no inferiority in the ocular pressure decrease of the preservative-free ophthalmic solution PRO-122, manufactured by Laboratorios Sophia S.A. de C.V., versus concomitant therapy in subjects with no-controlled primary open-angle glaucoma and/or intraocular hypertension" and number: SOPH122-0316/III do not have implications of BIOSAFETY, since no infectious or contagious biological material; pathogenic strains of bacteria or parasites; virus of any kind; Radioactive material of any kind; animals and/ or cells and/ or genetically modified plants; toxic, hazardous or explosive substances; any other material that may

endanger the health or physical integrity of the staff of the research center or research subjects or affect the environment will be used. In addition, it is stated that in this project transplant procedures of cells, tissues or organs, or cell therapy won't be carried out, neither laboratory animals, livestock nor wildlife will be used.

10.9 Final report and result publication

10.9.1. Final report

Once the statistical analysis is completed, a final report shall be drawn up with the obtained results, carried out by the Scientific Committee of the Department of Clinical Operations of Laboratorios Sophia, S.A. de C.V. Such report shall be drawn up following the recommendations of the ICH E3 guideline, Step 4.

10.9.2 Disclosure of results

Sponsor's plan to communicate the results of the study to the investigators, participants, and regulatory entities.

Regardless of the study outcomes, Laboratorios Sophia, S.A. de C.V., is committed to communicate the study final report to the principal investigators and the corresponding regulatory entities of the countries with participating research centers. Always maintaining the rights over the publication and disclosure of the information.

10.9.3 Publication of results

Laboratories Sophia, S.A. of C.V., serving as the study sponsor, assumes full responsibility for its function and retains the proprietary rights on the results of the study, which can be used in the manner it deems appropriate.

Since it is a multicenter study, the first publication must be made only with data collected from various centers and analyzed under the responsibility of Laboratorios Sophia, S.A. de C.V. The PI is committed to not publish or communicate the data collected just in one center or only in a part of the centers before the publication of the full results of the study, unless he/she has the prior written consent of Laboratorios Sophia, S.A. de C.V.

Any project of publication and/or communication related to the study and/or the results obtained during the study or after the completion of the study must be presented to the participating medical investigators at least 30 days in the case of a publication and 15 days in the case of a summary, before the scheduled date for communication and/or presentation of a publication. The medical investigator(S) will make their comments on the project within 15 days in the case of a publication and within 7 days in case of a summary, from the date the project is received.

However, if the sponsor is in the process of submitting a patent application on the study results, the sponsor may delay the publication or communication of the results of the study to date of registration.

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12. Signature page

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13. Annexes

13.1 Ocular comfort index

Ocular Comfort Index

Identification Sheet

Study No. SOPH122-0316/III **Date** / /

Subject Initials _____ **Subject No.** 122-____-____

Instructions:

This questionary was designed in order to score your eyes comfort.
Circle your answer in each question
Example: In the last week, ¿how often did your eyes were red?

Never						Always
0	1	2	3	4	5	6

There are not correct or incorrect answers. Do not take long in each question

1 In the last week, how often did your eyes feel dry?

Never						Always
0	1	2	3	4	5	6

When your eyes felt dry, tipically, how intense was the dryness?

Haven't felt it						Severe
0	1	2	3	4	5	6

2 In the last week, how often did your eyes feel gritty?

Never						Always
0	1	2	3	4	5	6

When your eyes felt gritty, tipically, how intense was the grittiness?

Haven't felt it						Severe
0	1	2	3	4	5	6

3 In the last week, how oftern did yur eyes feel stingy?

Never						Always
0	1	2	3	4	5	6

When your eyes stung, tipically, how intense was the stinging?

Haven't felt it						Severe
0	1	2	3	4	5	6

4 In the last week, how often did your eyes feel tired?

Never						Always
0	1	2	3	4	5	6

When your eyes felt tires, tipically, how intense was the tiredness?

Haven't felt it						Severe
0	1	2	3	4	5	6

Page 1 of 2

5 In the last week, how often did your eyes feel painful?

Never						Always
0	1	2	3	4	5	6

When your eyes felt painful, tipically, how intense was the pain?

Haven't felt it						Severe
0	1	2	3	4	5	6

6 In the last week, how iften did your eyes itch?

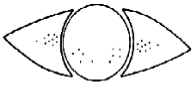
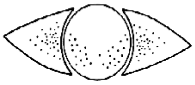
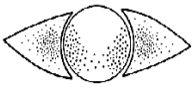
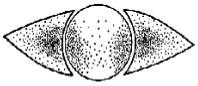
Never						Always
0	1	2	3	4	5	6

When your eyes itched, tipically, how intense was the itching?

Haven't felt it						Severe
0	1	2	3	4	5	6

13.2 Efron scale for conjunctival hyperemia

13.3 Oxford scale

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