Excelencia en oftálmicos

Statistical Analysis Plan

Phase I clinical study to evaluate the safety and tolerability of PRO-172 ophthalmic solution, manufactured by Laboratorios Sophia, SA de CV, on the ocular surface of ophthalmologically and clinically healthy subjects.

Creation date: September 2019.

Phase I clinical study to evaluate the safety and tolerability of PRO-172 ophthalmic solution, manufactured by Laboratorios Sophia, SA de CV, on the ocular surface of ophthalmologically and clinically healthy subjects.

Protocol Code: SOPH172-0919/I

Protocol Version: 0.1

Content

1.0 Abbreviations

FCI	Informed Consent Form
CRF	Electronic Case Report Form
EA	Adverse events
HC	Conjunctival hyperemia
ITT	Intention-to-treat population
LLS	Safety call
MAVC	Best corrected visual acuity
MC	Concomitant medication
ICO	Eye comfort index
PI	Research Product
PIO	Intraocular pressure
PP	Population by protocol
PRO-172	Bepostatin besylate 1.5%
TRL	Tear break-up time
VB	Initial visit
VF	Final visit

2.0 Objectives of the Study

To evaluate the safety and tolerability of the PRO-172 formulation manufactured by Laboratorios Sophia., SA de CV on the ocular surface of clinically healthy subjects.

3.0 Study Hypothesis

H_0: PRO-172 ophthalmic solution is safe and tolerable in its ophthalmic application, presenting in less than 10% of the study population unexpected adverse events related to the investigational product.

H_1: PRO-172 ophthalmic solution is not safe and tolerable for ophthalmic application, as it presented unexpected adverse events related to the investigational product in less than 10% of the study population.

4.0 Design of Study SOPH176-1218/I-II

Phase I clinical trial, controlled, non-comparative, open, single-center.

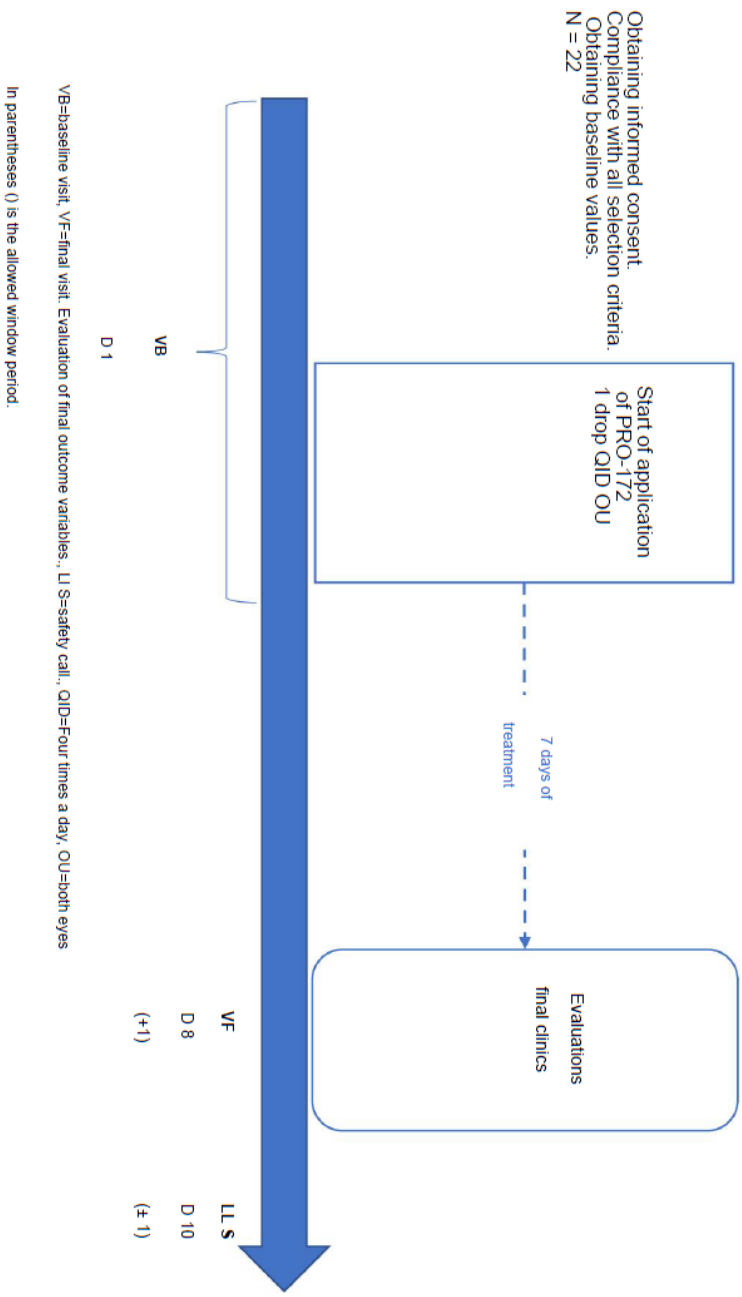
4.1 Duration of treatment

7 days

Table 1. Study schedule.

Procedures	VB D 1	VF D 8 TO +1	LIS D 10 ± 1
FCI SIGNATURE	X		
MEDICAL RECORD	X		
CONCOMITANT MEDICATION EVALUATION	X	X	
URINE PREGNANCY TEST	X	X	
VITAL SIGNS	X	X	
AVCC	X	X	
OCULAR SURFACE INTEGRITY (STAINING AND EVALUATION OF CONJUNCTIVAL HYPEREMIA AND CHEMOSIS)	X	X	

COMPREHENSIVE OPHTHALMOLOGICAL EVALUATION	X	X	
PIO	X	X	
ELIGIBILITY CRITERIA	X		
EA ASSESSMENT	X	X	X
RESEARCH PRODUCT (RP) ASSIGNMENT	X		
EYE COMFORT INDEX	X	X	
DELIVERY OF THE PI AND START OF INTERVENTION	X		
DELIVERY OF THE SUBJECT'S DIARY	X		
ADHERENCE ASSESSMENT		X	
RETURN/EVALUATION OF THE SUBJECT'S DIARY		X	
RETURN OF PI		X	



VB=baseline visit, VF=final visit. Evaluation of final outcome variables., LI S=safety call., QID=Four times a day, OU=both eyes

In parentheses () is the allowed window period.

Figure 1. Study diagram.

5.0 Sample Size

A total size of 22 subjects is estimated, who will provide both eyes for the analysis.

5.1 Sample Size Calculation

Although there are no references on sample calculation in phase I studies, it was considered pertinent to do so according to the presence of Adverse Events (AE) reported by Macejko et al., 2010 [1], in a multicenter, double-blind, randomized, parallel-group controlled clinical trial. Where the efficacy and tolerability of a 1.5% bepotastine besilate ophthalmic solution (n=43) versus 1.0% bepotastine (n=44) and placebo (n=43) were evaluated in 130 subjects in a conjunctival allergen challenge (CARC) model.

The intervention consisted of the application of 3 drops in each eye of each of the interventions (3 minutes after the RAC), with measurements at 15 minutes, 8 and 16 hours post-instillation.

The percentage of adverse events was 30% for the 1.5% bepostatin group, 47.5% for 1% bepostatin, and 22.5% for placebo, so a non-inferiority margin of 10% was considered with the formulation proposed in this protocol (1.5% bepostatin) [1].

The sample size was calculated using the equation for a proportion [2], considering a power of 80% (β), a significance level of 0.05 (α) and a non-inferiority margin (δ) of 10%.

Hypothesis

$$H_0: p - p_0 \leq \delta$$
$$H_1: p - p_0 > \delta$$

Where, δ is the non-inferiority margin (-10%).

Equation

The calculation to estimate the sample size and power was performed using the following equations.

$$n = p(1-p)\left(\frac{z_{1-\alpha} + z_{1-\beta}}{p - p_0 - \delta}\right)^2$$

$$1 - \beta = \theta(z - z_{1-\alpha}) + \theta(-z - z_{1-\alpha}) \, , z = \frac{p - p_0 - \delta}{\sqrt{\frac{p(1-p)}{n}}}$$

Where;

n is the sample size,

p_o is the reference proportion,

θ is the normal distribution function,

α is the Type I error

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

δ is the test margin.

Calculated sample: According to the previous calculation, the result is 18 subjects; this calculation was increased by 20% to account for possible losses. The total required sample size is 22 subjects, who will provide an input for the analysis.

6.0 Statistical Analysis Plan [3]

6.1 Primary outcome variables:

- Incidence of unexpected AEs related to the investigational product.
- ICO score.

6.2 Secondary outcome variables:

- Changes in Corrected Visual Acuity (CCVA).
- Corneal and conjunctival staining changes with lissamine green.
- Corneal and conjunctival staining changes with fluorescein.
- Changes in conjunctival hyperemia.
- Incidence of chemosis.

Table 3. Operational Definition of the Variables

Variable	Conceptual Definition	Operational Definition	Type of measurement	Normal value	Statistical test
Adverse events	Any adverse medical event that occurs in a patient or clinical research subject who has been administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. [4]	Adverse events occurring during the study will be collected using the electronic CRF.	• Continuous quantitative. • Qualitative categorical.	• Frequency: Subjects presenting AE/Total number of exposed subjects. • Intensity: 0= Mild 1= Moderate 2= Severe • Causality: 0=Probably or possibly related. 1=Unlikely related.	• X^2 or Fisher's Exacta.
Ocular Comfort Index (OCI) score.	The OIQ is a questionnaire designed to measure ocular surface irritation. It assesses symptoms focused on comfort associated with ocular surface disorders. Higher values indicate more severe symptoms.	The evaluator will administer the questionnaire to the subject and allow him or her to answer it calmly without any pressure or coercion. See Appendix 10.1, ICO .	Discrete quantitative.	Score: 0=None, 100=High discomfort.	• Wilcoxon rank test.
Changes in visual ability.	Best-corrected visual acuity (BCVA) is a test of visual function. Spatial VA is the ability to distinguish separate	Snellen chart	Discrete quantitative.	Fraction, normal value = 0.6 to 2.0	• Wilcoxon rank test.

	elements of an object and identify them as a whole. It is quantified as the minimum angle of separation (located at the nodal point of the eye) between two objects that allows them to be perceived as separate objects.				
Corneal and conjunctival staining changes with lissamine green.	Detection of epithelial defects in the conjunctiva and cornea.	Direct observation with a slit lamp, Oxford scale graduation. See Appendix 10.3, Oxford Scale .	Qualitative Ordinal	Degrees: The staining is presented in a series of panels (AE). The staining points range from 0-5 for each panel and from 0-15 for the total exposed area of conjunctiva and cornea.	• X^2 or Fisher's Exacta.
Corneal and conjunctival staining changes with fluorescein.	Detection of epithelial defects in the conjunctiva and cornea.	Direct observation with a slit lamp and cobalt blue filter, Oxford scale graduation. See Appendix 10.3, Oxford Scale .	Qualitative Ordinal	Degrees: The staining is presented in a series of panels (AE). The staining points range from 0-5 for each panel and from 0-15 for the total exposed area of conjunctiva and cornea.	• X^2 or Fisher's Exacta.

Conjunctival hyperemia changes.	It is defined as the simplest reaction of the conjunctiva to a stimulus; a red appearance is observed secondary to vasodilation of the vessels of the conjunctiva of variable intensity.	Direct observation. Classification using the Efron scale. See Appendix 10.2, Efron Scale for Conjunctival Hyperemia .	Ordinal qualitative.	Degrees: 0=Normal, 1= Very mild, 2= Mild, 3= Moderate, 4= Severe.	• X^2 or Fisher's Exacta.
Incidence of Chemosis.	It is defined as conjunctival edema resulting from an inflammatory reaction. It is classified as present or absent.	The evaluator will use a narrow beam of light at 60° and measure whether the conjunctiva separates from the sclera by ≥1/3 of the total eyelid opening or if it extends beyond the gray line.	Qualitative categorical.	0=Absent 1= Present	• X^2 or Fisher's Exacta.

7.0 Methods of Analysis [5]

Statistical analysis will be performed by personnel from Laboratorios Sophia, SA de CV. SPSS version 19.0 (IBM Corporation, Armonk, NY, USA) will be used. Coding will be performed using consecutive numbers. Data will be collected and organized in an Excel spreadsheet (Microsoft® Office). The data will then be exported to the SPSS platform. Variables will be categorized according to their nature (see Table 2).

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

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

Example:

A. A rieh Daniel M ercado C arrizalez B. J uan D e la Torre O rozco

a. Initials: AMC b. Initials: JDO

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

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

The Kolmogorov-Smirnov test will be performed to determine the distribution of the results obtained. [3]

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

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

- Intra-group analysis: They will be determined using the Wilcoxon rank test for quantitative variables. [6]

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

The results of the nominal and ordinal qualitative variables will be presented in frequencies, proportions and percentages.

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

- Intra-group analysis: Pearson's Chi-square (X^2) test or Fisher's exact test for expected values less than 5.

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

For adverse event reporting, all eyes of participants assigned to the intervention after the baseline visit will be considered. Results will be expressed as a percentage of subjects.

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

Table 3. Triangulation of concepts [5]

Variable type	Variable	A1	B1	B2	C1	C2	D1	D2	D3	D4
Background										
A1	Demographics	DT								
Basal										
B1	Medical record	DT								
B2	Comprehensive ophthalmological evaluation	DT			TB				B	
Security										
C1	EAs				TB	B	B	TB	TB	TB
C2	ICO	DB			B	B				
Secondary outcome										
D1	MAVC	DB			B		B			
D2	Ocular surface stains (TF and TVL).	DT			T			T		
D3	Conjunctival hyperemia	TD		DT	T				T	
D4	Chemosis	TD		DT	T					T

D, Descriptive statistics; T, 2x2 contingency table; B, Bivariate analysis; M, Multivariate analysis.

8.0 Changes

Newly created document, no changes apply.

8.1 Author of the document:

[redacted]

9.0 References

1. Macejko TT, Bergmann MT, Williams JKI., et al. Multicenter clinical evaluation of bepostatine besilate ophthalmic solutions 1.0% and 1.5% to treat allergic conjunctivitis. Am J Ophthalmol, 2010. 150(1): 122-7.

2. Chow S, Shao J, Wang H. Sample Size Calculations in Clinical Research. 2nd Ed. Chapman & Hall/CRC Biostatistics Series, 2008: Chapter 4: 85-6.

3. Haffajee A., Socransky S. and Lindhe J. Comparison of statistical methods of analysis of data from clinical periodontal trials. *J Clin Periodontol,* 1983; 10: 247-256.
4. Mexican Official Standard NOM-220-SSA1-2016, Pharmacovigilance facilities and operations.
5. ICH E2A. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonized Tripartite Guideline. Clinical safety data management: definitions and standards for expedited reporting. October 1994.
6. Woolson R. Wilcoxon signed-rank test. *Wiley Encuclopedia of Clinical Trials, 2008,* pp. 1-3.

10.0 Annexes

10.1 Eye comfort index

Eye Comfort Index

Identification card

Study No.: SOPH172-0919-I **Date:** / /

Subject's initials: **Subject No.:** 172-_____-_____

Directions:

This questionnaire was designed to rate the comfort of your eyes.

For each question, circle your answer.

Example: In the past week, how often were your eyes red?

Never						Always
0	1	2	3	4	5	6

There are no right or wrong answers. Don't spend too much time on each question.

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

Never						Always
0	1	2	3	4	5	6

When your eyes felt *dry*, how severe was the sensation usually?

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

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

Never						Always
0	1	2	3	4	5	6

When your eyes felt *gritty*, typically, how intense was the sensation?

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

3 In the past week, how often did your eyes feel *throbbing* ?

Never						Always
0	1	2	3	4	5	6

When your eyes felt like *they were stinging*, how intense was the sensation usually?

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

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

Never						Always
0	1	2	3	4	5	6

When your eyes felt *tired*, how intense was the feeling usually?

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

Sheet 1 of 2

Hoja 1 de 2

Eye comfort index

5 In the past week, how often did your eyes feel *sore* ?

Never						Always
0	1	2	3	4	5	6

When your eyes felt *sore*, how severe was the sensation usually?

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

6 In the past week, how often did your eyes feel *itchy* ?

Never						Always
0	1	2	3	4	5	6

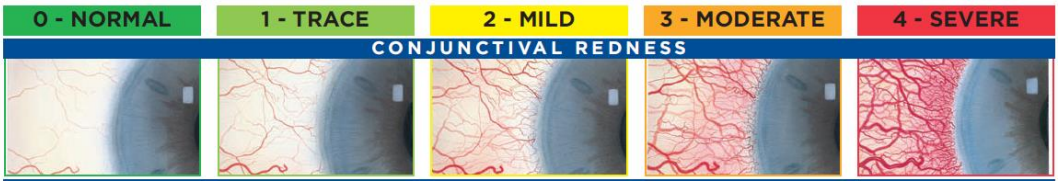
When your eyes felt *itchy* , how intense was the sensation usually?

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

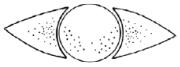
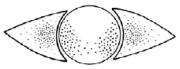
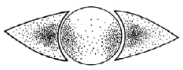
Ocular Comfort Index, translated from the Ocular Comfort Index available at: http:// iovs.arvohournals.org

Sheet 2 of 2

10.2 Efron scale for conjunctival hyperemia



10.3 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