Excelencia en oftálmicos

Statistical Analysis Plan

Phase I clinical study, to evaluate the safety and tolerability of PRO-165 ophthalmic gel versus Artelac® Nightime Gel, on the ocular surface of ophthalmologically and clinically healthy subjects.

Date of creation: July 2017.

Phase I clinical study, to evaluate the safety and tolerability of PRO-165 ophthalmic gel versus Artelac® Nightime Gel, on the ocular surface of ophthalmologically and clinically healthy subjects.

Protocol Code: SOPH165-0217/I.

Protocol Version: 2.0

Content

Abbreviations

BHc	Complete hematic biometry
CRF	Electronic Case Report Form
VA	Visual Ability
AE	Adverse events
HR	Heart rate
FCI	Informed Consent Form
FR	Respiratory rate
HC	Conjunctival hyperemia
ICO	Eye Comfort Index
ITT	Population by intent to treat
SC	Safety call
MC	Concomitant medication
PFH	Liver function tests
RP	Research Product
IOP	Intraocular pressure
PP	Population by protocol
PRO-165	Chondroitin sulfate 0.18%/Sodium hyaluronate 0.2%
BC	Blood chemistry
SBP	Systemic blood pressure
TBUT	Tear break-up time
BV	Initial visit
FV	Final visit

Objective of the Study

To evaluate the safety and tolerability of the PRO-165 formulation on the ocular surface of ophthalmologically and clinically healthy subjects.

Study Hypothesis

H0: PRO-165 Ophthalmic Gel has a similar safety and tolerability profile to Artelac® Nightime Gel in ophthalmologically and clinically healthy subjects.

H1: PRO-165 Ophthalmic Gel has a different safety and tolerability profile than Artelac® Nightime Gel in ophthalmologically and clinically healthy subjects.

Study Design SOPH165-0217/I

Phase I, controlled, parallel-group, double-blind, randomized, exploratory clinical trial.

Duration of treatment

Treatment: 10 days.

Study period: 4 months.

Table 1. Study timeline

Procedures	*Scrutiny*	Basal Visit	Visit 1	Final Visit	Call Security
	Day 0 - X[to]	Day 0	Day 5 ± 2	Day 11 to + 1	Day 13 ± 1
CI Signature	X				
Medical history	X				
Ophthalmological medical history	X				
Laboratory sample collection	X			X	
Review of Laboratory Tests		X			X
Pregnancy Test	X			X	
Eligibility Criteria	X	Xb			
Allocation		X			

Delivery of intervention		Xc			
Return of intervention				X	
Adherence assessment			X	X	
Adverse events			X	X	X
Intraocular pressure	X	1 x	X	X	
Visual Ability	X	1 x	X	X	
TRL	X	1 x	X	X	
Ocular surface stains	X	1 x	X	X	
Vitals	X	1 x	X	X	
Eye Comfort Index		X		X	
Subject Diary Delivery		X	X		
Return / Evaluation of the subject's Journal			X	X	
Return and Evaluation of the Quality Questionnaire				X	
Subject continuity assessment			X		

[a] The counting visit may be up to 10 days prior to the baseline, if it exceeds these the subject will not be able to enter.

[b] These criteria shall be supplemented by the results of laboratory examinations and those collected during the screening visit.

[c] An indication will be given to start the application the next day.

[1] They can be taken from the scrutiny visit, as long as no more than 10 days have elapsed, it is the prerogative of the PI to measure them again at the baseline visit.

Sample Size

An estimated 32 evaluable subjects are divided, divided into two groups [16 subjects (16 eyes) exposed per group].

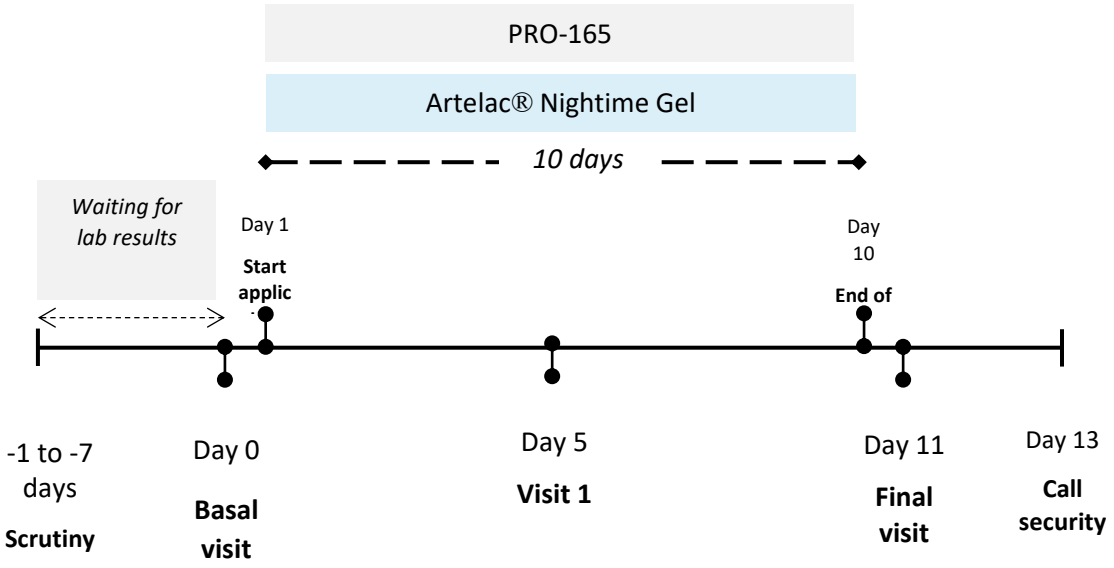

Figure 1. Suggested work scheme.

Sample Size Calculation

There are no references to sample size calculation in phase I studies, and the formulation of Humylub Ofteno® has not reported adverse events in its periodic safety reports. For this reason, it was considered pertinent to base it on the calculations of previous phase I studies, carried out by Laboratorios Sophia, S.A. de C.V., with formulations of ocular lubricants in the same pharmaceutical presentation.

The calculation was in accordance with the presentation of adverse events reported by Christ T (1994), in a randomized, single-blind, parallel-group controlled clinical trial where the efficacy and tolerance of a 5% dexpanthenol ophthalmic gel versus a 5% panthenol ointment in 48 subjects with corneal erosion was evaluated. keratoconjunctivitis from UV radiation or similar conditions . The intervention consisted of the application of 1 cm of the gel and ointment for 4 and 3 days, respectively. [1][2]

The percentage of good tolerance that was presented with the gel was 25%, so it is expected that no more than 10% of the subjects report a bad tolerance with the formulation proposed in this protocol.

The sample size was calculated using the formula for proportions.[3]

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

With a statistical confidence of 95% that corresponds to type I error, equal to 1.96, with a power of 90%, corresponding to type II error, equal to 0.84.

According to the previous calculation, the result is 26 subjects, which was increased by 20% due to the probable losses. The total sample size required is **32 subjects**. So each group will be made up of 16 subjects, who will provide an eye for the analysis.

Analysis Plan [4]

Outcome Variables

Safety primary outcome variables:

- Incidence of Adverse Events.

Primary outcome variables of tolerability:

- ICO score.

Secondary Outcome Variables:

- Tear film rupture time (LRT).
- Vital signs: HR, RF and SAP.
- Posterior segment.
- Quality questionnaire.
- Intraocular pressure.
- Visual capacity.
- Laboratory Tests: BHc, QS, PFH.
- Ocular surface stains.

Table 2. Operational Definition of the Variables

Variable	Conceptual Definition	Operational Definition	Measurement Type	Normal value	Statistical test
Adverse events (AEs).	Any undesirable medical event that may occur in an investigational subject during the clinical research stage of a drug or vaccine but does not necessarily have a causal relationship with it.[4]	Adverse events manifested during the conduct of the study will be collected using the CRF.	- Continuous quantitative. - Categorical qualitative.	- Frequency: Subjects with AEs/Total number of exposed subjects. - Intensity: 0= Mild 1= Moderate 2= Severe - Causality: 0= Improbably related. 1= Probable or possibly related. 2= Related	- Pearson's square chi or Fisher's exact. - U for Mann-Whitney.
Changes in Intraocular Pressure (IOP).	Tonometry is the objective measurement of IOP, based primarily on the force required to flatten the cornea or the degree of corneal indentation produced by a fixed force.	Goldmann tonometry. 3 samples will be taken, which will be recorded in the clinical file and the average will be recorded in the CRF.	- Continuous quantitative.	- 11-21 mmHg.	- U for Mann-Whitney. - Wilcoxon Ranges.
Eye Comfort Index (ICO) score.	The ICO is a questionnaire designed to measure irritation of the ocular surface, it assesses symptoms	The evaluator will apply the questionnaire to the subject and allow the subject to answer it calmly without any pressure and/or	Discrete quantitative.	Score: 0= None, 100 = High discomfort.	- U for Mann-Whitney. - Wilcoxon Ranges.

	focused on the comfort associated with ocular surface alterations. Elevated values indicate more Severe.	coercion. See ICO Annex.			
Changes in visual capacity (CV)	Visual acuity (VA) is a test of visual function. Spatial VA is the ability to distinguish separate elements of an object and identify them as a whole. It is quantified as the minimum angle of separation (located at the nodal point of the eye) between two objects that allows them to be perceived as separate objects.	Snellen's Primer	Discrete quantitative.	Fraction, normal value = 20/20	- U for Mann-Whitney. - Wilcoxon Ranges.
Changes in tear film rupture time (LRT) with fluorescein.	The stability of the tear film is usually evaluated in the clinic by the TRL. It consists of instilling fluorescein on the ocular surface to allow the visualization of the tear film	It will be measured at the end of a blink and will be asked not to blink immediately until the tear film on the cornea is broken.	Continuous quantitative.	> 10 seconds.	- U for Mann-Whitney. - Wilcoxon Ranges.

	and measure the time it takes to break since the last blink.				
Changes in corneal and conjunctival staining with lysmine green.	Detection of epithelial defects in the conjunctiva and cornea.	Direct observation with slit lamp, Oxford scale graduation. See annex Oxford scale.	Ordinal Qualitative	Degrees: The staining is presented by a series of panels (A-E). Stain points range from 0-5 for each panel and 0-15 for the total exposed area of the conjunctiva and cornea.	- Pearson's square chi or Fisher's exact. - McNemar test (if applicable).
Corneal and conjunctival staining changes with fluorescein.	Detection of epithelial defects in the conjunctiva and cornea.	Direct observation with slit lamp and cobalt blue filter, Oxford scale graduation. See annex Oxford scale.	Ordinal Qualitative	Degrees: The staining is presented by a series of panels (A-E). Stain points range from 0-5 for each panel and 0-15 for the total exposed area of the conjunctiva and cornea.	- Pearson's square chi or Fisher's exact. - McNemar test (if applicable).
Incidence of alterations in laboratory test results.	The normal parameters to be considered will be the ranges established by the laboratory, with a lower and upper margin of 20% in a clinically healthy subject, according to the PI's criteria.	Laboratory analysis.	Continuous quantitative.	See annex Scales to be used for vital signs and laboratory analysis.	- U for Mann-Whitney. - Wilcoxon Ranges.

Incidence of alterations in HR.	The normal parameters to be considered will be the ranges established by the laboratory, with a lower and upper margin of 20% in a clinically healthy subject, according to the PI's criteria.	Auscultation with a stethoscope.	Continuous quantitative.	60-100 lpm.	- U for Mann-Whitney. - Wilcoxon Ranges.
Incidence of alterations in RF.	The normal parameters to be considered will be the ranges established by the laboratory, with a lower and upper margin of 20% in a clinically healthy subject, according to the PI's criteria.	Auscultation with a stethoscope.	Continuous quantitative.	12-24 rpm.	- U for Mann-Whitney. - Wilcoxon Ranges.
Incidence of abnormalities in SBP.	The normal parameters to be considered will be the ranges established by the laboratory, with a lower and upper margin of 20% in a clinically healthy subject, according to the PI's criteria.	Measurement with sphygmomanometer.	Continuous quantitative.	< 120 / 80 mmHg	- U for Mann-Whitney. - Wilcoxon Ranges.

Methods of Analysis [4]

The statistical analysis will be carried out by personnel of Laboratorios Sophia, S.A. de C.V. The statistical program SPSS version 19.0 (IBM Corporation, Armonk, NY, USA) will be used. Designated personnel will be blinded to intervention groups. Coding will be performed using consecutive numbers for each intervention group.

The data will be collected and sorted in an Excel spreadsheet (Microsoft® Office). The data will then be exported to the SPSS program platform. The 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 study subject 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 compound surname, the first letter will always be used.

Example:

To. Arieh Daniel Carrizalez Market

to. Initials: AMC

B. Juan De la Torre Orozco

b. Initials: JDO

Once the subject has been selected, they will be assigned a number with which they will be identified throughout the study. This code will be made up of eight numbers in the following order from left to right:

- Three digits of the molecule under study according to the name by the sponsor.
- Two digits corresponding to the research centre number.
- Three digits of the number following their inclusion assigned to the research centre.

The results of the continuous quantitative variables will be presented in measures of central tendency: mean, standard deviation and ranges, see Table 2.

The Kolmogorov-Smirnov and Shapiro Wilk test will be performed to determine the type of distribution of the data (normality) in the results obtained in each study group. [5]

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

- Intra-group analysis: will be determined by Wilcoxon's rank test, for quantitative variables. [6]

- Inter-group analysis: Differences between groups will be analyzed using the Mann-Whitney U statistic.

The level of difference to consider significance will be an alpha (α) of 0.05 or less. A 95% CI will be considered for the non-inferiority criteria.

The result of the nominal and ordinal qualitative variables will be presented in frequencies, proportions and percentages, see Table 2.

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

- Intra-group difference: McNemar test. This is applied to 2×2 contingency tables with a dichotomous feature, with pairs of paired subjects, to determine if the marginal frequencies of row and column are equal (marginal homogeneity).

- Difference between groups: Pearson's Chi-square (*X2*) test or Fisher's exact test at expected values less than 5.

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

For the reporting of adverse events, all eyes of those participants who were randomly assigned to an intervention group after PI instillation (ITT) will be considered.

The final report of the results will be shown in tables or graphs, as appropriate.

Those subjects who meet a minimum adherence of 60% will be included in the statistical analysis to meet the objective of the study, taken from the subject's diary. However, even if the subject's adherence is greater than 60% per diary, if the adherence calculated by weight is less than 30%, the subject will not be included.

The investigational drug will be considered safe and tolerable when there are no clinical and statistical differences in all primary outcome variables, with respect to its comparator (Artelac® Nightime Gel).

Table 3. Triangulation of concepts

Variable Type	Variable	A1	B1	B2	C1	D1	E1	E2	E3	E4	E5	E6	E7	E8
Background														
A1	Demographic	DT												
Basal														
B1	Medical History	DT			T									
B2	Comprehensive ophthalmological evaluation	DT	DT		TB				TB		B	B		T
Safety														
C1	Incidence of AD			TB	TB		TB	TB	TB		TB	TB	TB	TB
Tolerability														
D1	ICO	DB				DB								
Secondary outcome														
E1	TRL	DB			TB		DB							
E2	Vitals	DB			TB			B						

E3	Posterior segment		DB	TB	B					
E4	Quality Questionnaire					TB				
E5	PIO		DB	TB			B			
E6	Visual Ability		DB	TB				B		
E7	Laboratory tests	DB		TB					B	
E8	Ocular surface stains		TB	TB						TB
D, Descriptive Statistics; T, 2x2 contingency table; B, Bivariate Analysis; M, Multivariate Analysis.										

Changes

1. First version of the document, no changes apply.

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3. Chow S, Wang H, Shao J. Sample size calculation in Clinical Research. New York: Taylor Francis, 2007.

4. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Clinical Safety Data Management: Definitions and Standards for Expedited Reporting E2A. ICH Harmonised Tripartite Guideline, Vol. 4, 1994.

5. Haffajee A, Socransky S, Lindhe J. Comparison of satatistical methods of analysis of data from clinical periodontal trials. J Clin Periodontol, 1983, 247-56.

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Annexes

Eye Comfort Index

Eye Comfort Index

Identification card

Study No.: SOPH172-0913-I

Subject's initials: _____

Date: / /

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

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

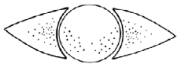
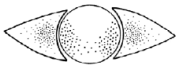
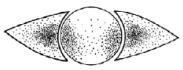
6 In the past week, how often did your eyes feel *itchy* ?

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

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