Creation date: January 2019.

Phase I-II clinical study to compare the safety and efficacy of Nanodrop® versus Systane® Balance in the treatment of patients with dry eye.

Protocol Code: SOPH176-1218/I-II

Protocol Version: 2.0

Content

1.0 Abbreviations

CI	Informed Consent
CRF	Electronic Case Report Form
EA	Adverse events
ITT	Intention-to-treat population
LLS	Safety call
MAVC	Best corrected visual acuity
MC	Concomitant medication
OSDI	Ocular Surface Disease Index
PI	Research Product
PP	Population by protocol
PRO-176 Nanodrop®	
TRL	Tear break-up time
V1	Follow-up visit 1
VB	Initial visit
VF	Final visit

2.0 Objectives of the Study

Security (Phase I):

To evaluate the safety of ophthalmic application of Nanodrop® by quantifying the incidence of unexpected Adverse Events (AEs) related to the investigational product (IP).

Efficacy (Phase II):

To demonstrate the non-inferiority of Nanodrop® compared to Systane® Balance in the treatment efficacy of patients with dry eye, using the OSDI (Ocular Surface Disease Index) test score.

3.0 Study Design SOPH176-1218/I-II

Phase I-II, comparative, non-inferiority, active-controlled, parallel-group, double-blind, randomized clinical trial. Safety analysis comparing the visits of the first 12 patients in the Nanodrop® group. If fewer than 20% of unexpected adverse events (AEs) related to the investigational product occur, recruitment continues until the sample for the efficacy analysis is complete.

3.1 Duration of treatment

30 days.

Table 1. Study schedule

Procedures	VB	V1	VF	LIS
	D 1	D 8	D 31 to + 1	D 37 ± 1
CI Signature	X			
Medical record	X			
MC Evaluation	X	X	X	
Urine pregnancy test	X		X	
MAVC	X	X	X	
Ocular surface stains	X	X	X	
Tear film break-up time with fluorescein	X	X	X	
Ophthalmological evaluation comprehensive	X	X	X	
Eligibility criteria	X			
EA Assessment	X	X	X	X
PI Assignment	X			
Delivery of PI and start of intervention	X			
Delivery of the PI and start of intervention	X			
Delivery of patient materials and instructions for completion (Subject Diary)	X			
Evaluation of the Diary of the subject		X	X	
Adherence assessment		X	X	
Continuity assessment of the subject		X	X	
Return of PI and Subject's Diary			X	

CI: Informed Consent; BCVA: Best Corrected Visual Acuity; OSDI: Ocular Surface Disease Index; AE: Adverse Event; PI: Investigational Product; MC: Concomitant Medication.

4.0 Sample Size

An estimated 63 subjects (both eyes) per treatment arm are planned (Phase I: 12 subjects / Phase II: 114 subjects).

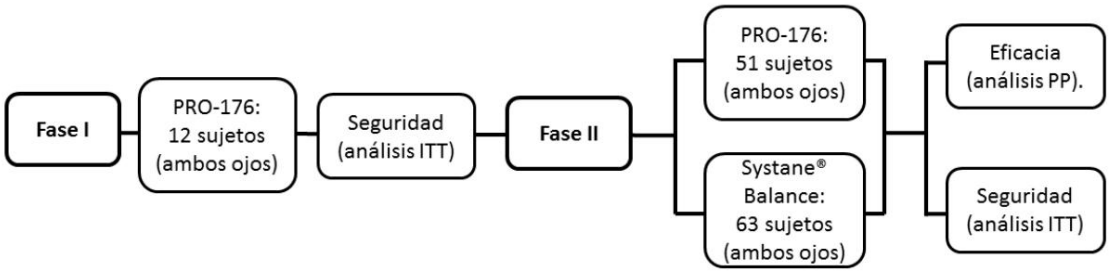

Figure 1. Suggested workflow. ITT: Intention-to-treat population; PP: Per-protocol population.

5.0 Sample Size Calculation

For the sample size calculation, changes in the OSDI *(Ocular Surface Dehydration Index) questionnaire score were considered,* in studies with ocular lubricants in patients with dry eye disease (DED), as the primary efficacy variable.

PRO-176 (Nanodrop®; Laboratorios Sophia, SA de CV) is expected to be non-inferior to its comparator (Systane® Balance; Alcon Research Ltd, Fort Worth, TX, USA), according to the following working hypotheses:

$$H_0: \varepsilon \leq \delta$$
$$H_1: \varepsilon > \delta$$

Where ' $\mu_A - \mu_B = \varepsilon$,' is the true difference in means between the test treatment (PRO-176) and the active control (Systane® Balance). Where δ is the non-inferiority margin, and the ratio of the sample sizes between the two groups is given by:

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

Calculations were performed using the non-inferiority equation for two means and for statistical power (Chow et al., 2008).

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

$$1 - \beta = \theta(z - z_{1-\alpha}) + \theta(-z - z_{1-\alpha}) \;,\; z = \frac{\pi_A - \pi_B - \delta}{\sigma\sqrt{\frac{1}{n_A} + \frac{1}{n_B}}}$$

Where σ is the standard deviation, k=nA/nB is the agreement ratio, θ is the standard Normal distribution function, α is the type I error (confidence interval), β is the type II error (meaning that $1-\beta$ corresponds to the power) and δ is the test margin.

With a power of 80% (β=0.20), a significance level of 0.05 (α) and a non-inferiority margin (δ) of -5 points on the OSDI score (Grubbs et al., 2014; Labetoulle et al., 2017; Pérez-Balbuena et al, 2016).

The calculation was based on the study by Benitez-Del-Castillo et al., 2016. Phase I and II clinical study. A total of 156 healthy subjects and patients with DED were enrolled. The Phase I study included 30 healthy volunteers; it was a single-center, parallel-group, open-label study. Phase II was a multicenter, double-blind study. In Phase I, SYL1001 was administered at 1.125% and 2.25% doses, and in Phase II, at 0.375% and 0.75%. After 10 days of treatment, the primary efficacy variable was the effect on the OSDI score. OSDI scores were significantly reduced compared to baseline ($p<0.01$) for all treatments in both Phase I and Phase II. No statistical differences were observed between treatments.

The sample size calculation was performed considering a reduction in the OSDI score of 17.6 points for the placebo group at their final visit versus a reduction of 16.5 points for the treated group. Considering a ÿ of 17.1, a similar reduction in the OSDI score is expected for both treatments, with no statistically significant differences at their final visit.

The calculation was performed using the online tool: http://powerandsamplesize.com.

The estimated sample size was 101 subjects (51 subjects per arm), which was increased by 25% to account for potential losses (25 subjects). The estimated number of subjects (both eyes) was 126.

6.0 Analysis Plan

The variables to be considered in the protocol are described below.

Primary efficacy outcome variables:

- OSDI test score.

Secondary efficacy outcome variables:

- Changes in best corrected visual acuity (BCVA).
- Changes in corneal and conjunctival staining with lissamine green.
- Corneal and conjunctival staining changes with fluorescein.
- Changes in tear film break-up time (TRL) with fluorescein.

Security Variables

Primary Safety outcome variable:

- Incidence of unexpected adverse events (AEs) related to the drug.

Secondary safety outcome variable:

- Incidence of expected AEs.

Table 2. Operational Definition of the Variables

Variable	Definition Conceptual	Definition Operational	Scale of measurement	Category
Events adverse	Any event adverse doctor that occurs in a patient or clinical research subject who was administered a pharmaceutical product and that, not necessarily has a relationship causal with this treatment.	The events adverse effects manifested during the conduct of the study will be collected through the electronic CRF.	• Nominal • 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.
Ocular Surface Disease Index Score (OSDI).	The OSDI is a questionnaire designed to establish a severity and classification of the dry eye according to its symptoms.	The evaluator will apply the questionnaire to the subject and allow him to do so answer in calm without any kind of pressure and/or coercion. See Annex 1. OSDI.	Continue	Score: $OSDI = \frac{(Suma\ del\ puntaje)25}{\#\ de\ respuestas\ contestadas}$
Changes in corrected visual acuity (BCVA).	Visual acuity corrected (BCVA) 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	Booklet of Snellen	Quantitative discreet.	Fraction, normal value = 0.6 to 2.0

	It quantifies 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.			
Changes of corneal and conjunctival staining with green lissamine.	Detection of epithelial defects in the conjunctiva and cornea.	Observation direct with slit lamp, Oxford scale graduation. See Annex 2. 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.
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 2. 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.
Changes in tear film break-up time (TRPL) with fluorescein.	The stability of the Tear film is usually evaluated clinically by the TRL. This involves instilling fluorescein on the ocular surface to allow visualization of the tear film and measure the time it takes for the tear film to break up after the last blink.	It will be measured at end of a blink and you will be asked not to blink immediately until the tear film on the cornea breaks.	Quantitative continues.	Normal: > 10 seconds.

7.0 Methods of Analysis

Statistical analysis will be performed by staff of Laboratorios Sophia, SA de CV. The statistical program SPSS version 19.0 (IBM Corporation, Armonk, NY, USA) will be used.

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

The data will be collected and organized in an Excel spreadsheet (Microsoft® Office). The data will then be exported to the SPSS software 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 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:

TO.	Arieh Daniel Mercado Carrizalez	B. Juan De la Torre Orozco
to.	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 sponsor's designation. • Two digits corresponding to the research center number. • Three digits of the consecutive number assigned to its inclusion at the research center.

Subjects will be divided into two equal groups, which will be randomly and blindly assigned to one of the PI, PRO-176 or Systane® Balance (assignment (1:1). Which will be administered based on the dosage described in the protocol.

A preliminary safety analysis will be performed upon completion of follow-up of subject 12 in the Nanodrop® group (Phase I).

If fewer than 20% of unexpected drug-related AEs occur in the Nanodrop® group, enrollment for the efficacy analysis (Phase II) will be completed. Otherwise, the study will be terminated.

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 whether the distribution is normal in the results obtained in each study group (Haffajee et al., 1983).

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

- Intra-group analysis: will be determined using the Wilcoxon rank test, for quantitative variables (Woolson, 2008).
- Between-group analysis: differences between groups will be analyzed using the Student t-test or the Mann-Whitney U statistic if applicable (it will be used to test whether a group of data comes from the same population).

The level of difference to consider significance will be an alpha (ÿ) of 0.05 or less. It will be considered a 95% CI for non-inferiority criteria (Schumi & Wittes, 2011; Easty et al., 2006).

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

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 difference: McNemar's test (Klingenberg & Agresti, 2006). This test is applied to 2x2 contingency tables with a dichotomous trait, with matched subject pairs, to determine whether the marginal frequencies of the row and column are equal (marginal homogeneity).
- Difference between groups: Chi-square test *(X^2)* Pearson's or Fisher's exact test in values expected under 5.
-

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

For the reporting of adverse events, all eyes of participants who were randomized to an intervention group after the baseline visit will be considered.

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

Subjects who meet a minimum adherence of 70% will be included in the statistical analysis to meet the study objective, based on the weight of the PI. In cases where the container is not returned or has not been physically intact, adherence will be measured using the subject's diary.

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

Table 3. Triangulation of concepts

Variable Type	Variable	A1	B1	B2	C1	C2	D1	E1	E2	E3
Background										
A1	Demographics	DT								
Basal										
B1	Medical record	DT								
B2	Comprehensive ophthalmological evaluation	DT		TB	TB				TB	
Security										
C1	Unexpected/related AEs		TB		TB			TB	TB	TB
C2	EAs		TB			TB		TB	TB	TB
Effectiveness										
D1	OSDI		DB				DB			
Secondary outcome										
E1	MAVC			DB	TB	TB		DM		
E2	Ocular surface stains			DB	TB	TB			DM	
E3	Tear film time			DB	TB	TB				DM

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

8.0 Changes

The following numerals were changed to match the current version of the protocol:

- 2.0 Study objectives (Effectiveness; phase II).
- 3.0 Study design.
- Table 1. Study schedule.
- 5.0 Sample size calculation.
- 7.0 Methods of analysis, referring to adhesion.

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9.0 References

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10.0 Annexes

10.1 Ocular Surface Disease Index

The OSDI (ocular surface disease index) test is a simple test designed to establish the severity and classification of dry eye based on its symptoms.

Please answer the following questions by checking the box that best represents your answer:

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

	FREQUENCY				
	Throughout moment	Almost in everything moment	50% of the time	Almost in no moment	In no case moment.
Light sensitivity.	4	3	2	1	0
Gritty feeling in the eyes.	4	3	2	1	0
Eye pain	4	3	2	1	0
Blurred vision.	4	3	2	1	0
Poor vision.	4	3	2	1	0
				Subtotal:	

2. Have you had eye problems that have limited or prevented you from performing any of the following actions? during the last week?

	FREQUENCY				
	Throughout moment	Almost in everything moment	50% of the time	Almost in no moment	In no case moment.
Read.	4	3	2	1	0
Driving at night.	4	3	2	1	0
Working on a computer or using an ATM.	4	3	2	1	0
Watch television.	4	3	2	1	0
				Subtotal:	

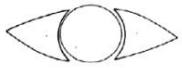
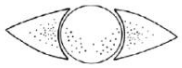
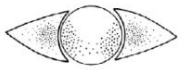
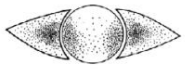
3. Have you experienced eye discomfort in any of the following situations during the last week?

	FREQUENCY				
	Throughout moment	Almost in everything moment	50% of the time	Almost at no time	Not at any time.
Wind.	4	3	2	1	0

Low humidity (dry) places.	4	3	2	1	0
Areas with air conditioned.	4	3	2	1	0
				Subtotal:	

$$= \frac{(\qquad\qquad)25}{\#}$$

10.2 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