Clinical Investigation Plan

"MULTICENTRIC CLINICAL INVESTIGATION ON THE USE OF SINGLE-DOSE OPHTHALMIC SOLUTION BASED ON SODIUM HYALURONATE IN THE TREATMENT OF EYE *DISCOMFORT* IN PARTICULAR IN CASE OF OCULAR DRYNESS"

Version 2 March 14th, 2023

STUDY CODE: 052/SI Hyalistil Bio PF Mono

Post Market Clinical Follow up (PMCF)
(Observational Study-Profit)

Clinical Investigation Plan Status: FINAL
Clinical Investigation Plan No.: 052/SI Hyalistil Bio PF Mono
Study Type: Post Market Clinical Follow up (PMCF) (Prospective, observational, non-interventional clinical investigation)
Products Name: Hyalistil Bio PF
Date of Issue: *March 14th, 2023*
Amendment: 052/SI Hyalistil Bio PF Mono/EM version 1 of March 14th,2023
Study Title Multicentric clinical investigation on the use of single-dose ophthalmic solution based on sodium hyaluronate in the treatment of eye *discomfort* in particular in case of ocular dryness
Sponsor SIFI SpA Via Ercole Patti 36 95025 Aci S. Antonio (CT) – (Italy) Dr. Fabrizio Chines Tel: +39 0957922136 Mob: +39 3389472159 Email: fabrizio.chines@sifigroup.com
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Principal Investigators **Dr. Davide Scollo** Oculistica, P.O. San Marco, Azienda Ospedaliero Universitaria Policlinico "G.Rodolico - San Marco" Viale Carlo Azeglio Ciampi, 95121 Catania (Italy) Tel: +39 095378121 Email: davidescollo@hotmail.com **Prof. Vincenza Maria Elena Bonfiglio** U.O. di Oculistica, Presidio Belmonte, Azienda Ospedaliero Universitaria Policlinico "Paolo Giaccone" Via Rampolla Mariano Cardinale, 10B, 90142 Palermo (Italy) Tel: +39 091 6553903 Email: enzabonfiglio@gmail.com

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APPROVAL SHEET

I, here undersigned, approve the content of the Clinical Investigational Plan.

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I, here undersigned, approve the content of the Clinical Investigational Plan.

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I, here undersigned, approve the content of the Clinical Investigational Plan.

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Signature Date

INVESTIGATOR SIGNATURE

I have read and understood this Clinical Investigation Plan. I will conduct the study in accordance with all applicable national and regional laws and regulations and will strictly follow the study procedures.
I agree to provide all the information requested in the electronic Case Report Forms.
I also agree that all information provided to me by the Sponsor (or Sponsor's designees), including clinical data, clinical investigation plans, Case Report Forms, and any verbal and written information, will be kept strictly confidential. It is recognized that this information may be relayed in confidence to the Ethics Committee or regulatory authorities.
In addition, no reports or information about this observational study will be provided to anyone not involved in the study other than the Sponsor (or Sponsor's designees), the Ethics Committee(s) or regulatory authorities. Any such submission will indicate that the material is confidential.

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CHANGE HISTORY RECORD

Clinical Investigation Plan version	Clinical Investigation Plan date	Description of changes	Amendment version	Amendment date
1	October 10th 2022	First release	-	-
2	March 14th, 2023	Change on: Primary objective Statistical Analysis Number of Box to delivery Procedures	1	March 14th, 2023

CLINICAL INVESTIGATION PLAN SYNOPSIS

TITLE	Multicentric clinical investigation on the use of single-dose ophthalmic solution based on sodium hyaluronate in the treatment of eye *discomfort* in particular in case of ocular dryness
CLINICAL INVESTIGATION CODES	052/SI Hyalistil Bio PF Mono
AMENDMENT	052/SI Hyalistil Bio PF Mono/EM version 1 of *March 14th,2023*
AIM OF THE CLINICAL INVESTIGATION	To assess the performance and safety resulting from the treatment of an ophthalmic solution for the ocular *discomfort* and in particular in presence of ocular dryness.
CLINICAL INVESTIGATION SITES	1) P.O. San Marco, Azienda Ospedaliero Universitaria Policlinico "G. Rodolico - San Marco" Oculistica, Viale Carlo Azeglio Ciampi, 95121 Catania (Italy) **Dr. Davide Scollo** Tel: +39 0953781213 Email: davidescollo@hotmail.com 2) U.O. di Oculistica, Presidio Belmonte, Azienda Ospedaliero Universitaria Policlinico "Paolo Giaccone" Via Rampolla Mariano Cardinale, 10B, 90142 Palermo (Italy) **Prof. Vincenza Bonfiglio** Tel: +39 091 6553903 Email: enzabonfiglio@gmail.com
CLINICAL INVESTIGATION PHASE	Post Market Clinical Follow up (PMCF) (Investigator sponsored per profit, prospective, observational, non-interventional clinical investigation)
CLINICAL INVESTIGATION PERIOD	Approximately 4 months from first patient first visit (FPFV) to last patient last visit (LPLV) Each patient enrolled will be treated for 35± 4 days. Enrollment period: 3 months.
CLINICAL INVESTIGATION DESIGN	Multicenter, prospective, observational, open-label, non- interventional clinical investigation evaluating the performance and safety of 4 daily instillations of the ophthalmic solution in the treatment of mild irritation of the ocular surface.
CLINICAL	28 male and female subjects with signs and symptoms of ocular

INVESTIGATION POPULATION	*discomfort* and ocular irritation resulting from ocular dryness who qualifies for Hyalistil Bio PF treatment.
NUMBER OF SUBJECTS	28 subjects recruited with competitive enrolment at the involved study sites.
SUBJECT SELECTION CRITERIA	***Inclusion criteria*** 1. Subjects (male or female) must be ≥ 18 years of age; 2. Subject able to provide Informed Consent, in compliance with the good clinical practice and local laws (thus, subject able to comprehend the full nature and purpose of the study, including possible risks and side events); 3. Subjects with ocular discomfort resulting from mild to moderate dry eye in one or both eyes determined by: - Scoring of ocular surface staining with fluorescein using the National Eye Institute (NEI) scale. Total score per single eye range 6-33 summing the score of cornea and conjunctiva. (Considering a normal score of 0-33); - Tear film break-up time with fluorescein (TFBUT) ≤ 10 seconds; The TFBUT value will be recorded as the average of 3 measurements; - Symptom Assessment in Dry Eye (SANDE) questionnaire ≥ 35. 4. Subject able to be compliant with the requirements of the clinical investigation plan, according to the Investigator; 5. Subject who qualifies for Hyalistil Bio PF treatment according to the approved indication; 6. Subject who in physician's opinion will benefit from this treatment. ***Exclusion criteria*** 1. Corneal injuries or abrasions of traumatic origin in the eye of study; 2. Ocular infection or clinically significant inflammation (such as Herpes Simplex infection, corneal virus infection, bacterial, viral or fungal conjunctivitis, tuberculosis and mycosis of the eye, purulent and herpetic blepharitis, stye); 3. Sjögren's syndrome; 4. Stevens-Johnson syndrome; 5. Systemic lupus erythematosus; 6. Pathologies associated with corneal thinning; 7. Taking drugs that may interfere with tear gland secretion (beta - blockers); 8. Patients using any topical therapies such as non-steroidal anti-inflammatory drugs, cortisone, cyclosporine, vasoconstrictor, artificial tears (different than the investigated product) the eye of study;

	9. Treatment with topical or systemic corticosteroids in the 4 weeks preceding the study; 10. Known or potential hypersensitivity and/or history of allergic reactions to one of the components of the topical medical device. 11. Participation in another clinical trial within the previous 30 days; 12. Evidence of severe or uncontrolled systemic disease or any other significant disorders, which in the opinion of the Investigator does not allow the participation in the study or could compromise the results.
CLINICAL INVESTIGATION OBJECTIVES	***PRIMARY OBJECTIVE*** • Evaluation of Tear film break-up time with fluorescein (TFBUT) at Study Termination Visit (*Day 35 ± 4 of treatment*) compared to Visit 1 (*Day 0 of treatment - baseline*). ***SECONDARY OBJECTIVE*** • Evaluation of the change in total score (score from 6 to 33, considering a normal score 0-33) resulting from the sum of the corneal staining score (score from 0 to 15) and conjunctival staining score (score from 0 to 18) with fluorescein using the National Eye Institute Scale (NEI) at Visit 2 *(Day 14 ± 2 of treatment)* and at the Study Termination Visit *(Day 35 ± 4 of treatment)* compared to Visit 1 *(Day 0 of treatment - baseline);* • To evaluate the tear film stability per group as objectified by the tear break up time with fluorescein (TFBUT) at Study Termination Visit *(Day 35 ± 4 of treatment)* compared to Visit 2 (*Day 14 ± 2 of treatment*). • Changes about Best Corrected Visual Acuity (BCVA) measured by the "*Early Treatment Diabetic Retinopathy Study*" (ETDRS) at Visit 2 (*Day 14 ± 2 of treatment*) and Study Termination Visit *(Day 35 ± 4 of treatment)* compared to Visit 1*(Day 0 of treatment – baseline)*. • Patient reported outcomes: To compare patients reported outcomes (PRO) measures per group, including: - Patient's reported symptoms (SANDE) at Visit 2 (*Day 14 ± 2 of treatment*) and Study Termination Visit *(Day 35 ± 4 of treatment)* compared to Visit 1*(Day 0 of treatment – baseline)*. - Assessment of the quality of life (QOL)### by "*Questionnaire about Eye Symptoms and Daily Life*" (DEQS) at Study

	Termination Visit (*Day 35 ± 4 of treatment*) compared to Visit 1 (*Day 0 of treatment - baseline*). - Evaluation of the degree of satisfaction to the treatment reported by patients through the use of the visual analogue scale (VAS)[##], at the Study Termination Visit (*Day 35 ± 4 of treatment*). [###]In the event that, for both trial centers, the DEQS questionnaire is not available, the assessment of the quality of the life will be performed by the DEQ-5 questionnaire. ***SAFETY EVALUATIONS*** • Evaluation of the safety and the *compliance* of medical device.
ENDPOINTS^	***PRIMARY ENDPOINTS[#]*** • *Clinical performance:* - 25 ~~30~~% increase from Visit 1 (*Day 0 of treatment - baseline*) to Study Termination Visit (*Day 35 ± 4 of treatment*) of tear film break-up time with fluorescein (TFBUT)[ϕ]. [Time frame: Visit 1 and Study Termination Visit]. ***SECONDARY ENDPOINTS*** • Reduction from baseline (*Visit 1- Day 0 of treatment*) in the corneal and conjunctival fluorescein staining score using the NEI scale. Fluorescein staining of the ocular surface is assessed as the total sum of the corneal and conjunctival subregions. - Corneal Fluorescein Staining (NEI score, sum of 5 subregions, maximum score 15. Each subregion will have a maximum score of 3. (0=no staining, 3 = maximum staining). - Conjunctival Fluorescein Staining (NEI score, sum of 6 subregions, maximum score 18. Each subregion will have a maximum score of 3. (0=no staining, 3 = maximum staining). [Time frame: Visit 1, Visit 2 and Study Termination Visit]. • Tear film break-up time with fluorescein (TFBUT)[ϕ]. [Time frame: Visit 2 and Study Termination Visit]. • BCVA is measured using the standard Early Treatment Diabetic Retinopathy Study (ETDRS) acuity chart and reported in ETDRS letters. [Time frame: Visit 1, Visit 2 and Study Termination Visit]; • *Patient reported outcomes:* - Change in intensity and frequency of dry eye symptoms assessed by completing the questionnaire SANDE. [Time frame: Visit 1, Visit 2 and Study Termination Visit]. - [##]Evaluation of the degree of satisfaction to the treatment reported by patients through the use of the visual analogue scale (VAS) [##], ranging from 0 to 100 mm, at Study Termination Visit (*Day 35 ± 4 of*

treatment). [Time frame: Study Termination Visit].

Patients will be asked to rate their degree of satisfaction to the treatment and answer the following questions:

- "I feel satisfied using this treatment? ",
- "With this treatment, I have a feeling of freshness?",
- "With this treatment, I have a feeling of relief?",
- "This treatment contributed to reduce my pain due to eye dryness?",
- "This treatment is comfortable? ",

Patients will be asked to place a vertical mark on a horizontal line, from 0 to100 mm, to indicate the degree of satisfaction to the treatment, where:

✓ 0-10 mm=none
✓ 11-30 mm=very mild
✓ 31-50 mm=mild
✓ 51-70 mm=moderate
✓ 71-90 mm=strong
✓ 91-100 mm=very strong.

-Assessment of the quality of life (QOL) by "*Questionnaire about Eye Symptoms and Daily Life*" (DEQS)### at Study Termination Visit (*Day 35 ± 4 of treatment*) compared to Visit 1 (*Day 0 of treatment - baseline*). [Time frame: Visit 1 and Study Termination Visit].

###In the event that, for both trial centers, the DEQS questionnaire is not available, the assessment of the quality of the life will be performed by the DEQ-5 questionnaire.

EVALUATION OF SAFETY PARAMETERS

✓ Evaluation of safety of daily instillation of ophthalmic solution during all the study period through:

- Investigator Global Assessment of Safety (IGAS): using the 4-point scale:1= very good safety, 2 =good safety, 3 = moderate safety and 4 = poor safety. IGAS will be evaluated at the Study Termination Visit. [Time Frame: Study Termination Visit];
- Evaluation of reported adverse events/incidents. [Time Frame: During the treatment period].
- Evaluation of intraocular pressure (IOP). [Time frame: Visit 1, Visit 2 and Study Termination Visit];
- Evaluation of *compliance* through verification of correct instillation of the medical device, counting of single dose containers and boxes. [Time Frame: Visit 2 and Study Termination Visit].

^According to clinical practice, the Investigator will perform all clinical

	evaluations on both eyes of the individual patient. In the case that both eyes are affected by ocular dry eye, the statistical analysis will be performed considering the eye with the lowest level of TFBUT score "worse eye". If the level of TFBUT score at baseline is the same in both eyes, then the eye with the highest level of NEI score will be designated as the "worse eye." If the level of NEI score at baseline is the same in both eyes, then the right eye will be designated as the "worse eye." #The primary end point will be evaluated on the PP population and also in ITT population.
STATISTICS	**Sample size estimate** A previous study report baseline level of tear film break-up time with fluorescein (TFBUT) of 6.23±1.75 at baseline, to detect a 25 increase of TFBUT at the Study Termination Visit compared to baseline (Visit 1) (mean difference 1,56, sd 1.91), with an of alpha=0.01(two-tailed) and a power (1 – ß) of 0.80, 23 subjects are requires (Wilcoxon signed-rank test). Considering a dropout of 20%, it is expected to enroll a total of 28 subjects. **Statistical analysis** Descriptive analysis will be performed by using absolute rate, percentage and frequency tables for qualitative variables. Normally distributed data will be reported as minimum, maximum, means and standard deviations, whereas non- normally distributed data as medians and interquartile range (IQR). To compare qualitative variables for not-paired data will be used the Chi-square test with Yates corrections or Fisher's exact test when appropriate and the Mc Nemar's test for paired data. Differences in endpoints between baseline and different timepoints will be assessed using paired t-test for normally distributed data, whereas the Wilcoxon signed-rank test will be used for non- normally distributed data. Data distribution will be tested by using the Kolmogorov-Smirnov test. A p-value <0.05 will be considered statistically significant. All the Analysis will be performed using SAS version 9.4 for Windows (SAS Institute Inc., Cary, NC, USA). **Analysis set** All patients enrolled will be included in the safety population. All patients enrolled having a primary post-baseline -performance assessment will be included in the ITT population. If the primary performance measurement at Visit 2 is missing, it will be imputed with the score at baseline. If the primary performance

	measurement at Study Termination Visit is missing, it will be imputed with the score at Visit 2, if it is not missing, or otherwise with the score at baseline. All patients of the ITT population with any major clinical investigation plan deviation will be included in the PP population. The analysis of safety endpoints will be performed in the safety population. Analysis of performance endpoints will be performed on the ITT population. The analysis of the primary endpoint will be performed on the PP population but will be also repeated in the ITT population.
MEDICAL DEVICE	Hyalistil Bio PF
ROUTE OF ADMINISTRATION AND DOSAGE	1 drop of Hyalistil Bio PF in the conjunctival fornix, 4 times a day. Hyalistil Bio PF can also be used by contact lens wearers. It is not necessary to remove contact lenses before administering Hyalistil Bio PF. Moreover, advised not to administer other drugs or medical devices for ocular use within 10 minutes from the instillation of Hyalistil Bio PF
INVASIVE PROCEDURES	There are no invasive techniques outside of normal clinical practice for the purposes of the study. Regarding Tear Film Break-Up Time test with fluorescein (TFBUT), fluorescein staining is an invasive procedure and is normally used in clinical practice in the assessment of ocular discomfort due to dry eye.
CHRONOGRAM OF VISITS AND EVALUATIONS	**<u>Visit 1 (screening-enrolment)</u>** The following procedures will be performed: - Explanation to the patient of study aims, procedures and possible risks of the observational study; - Informed consent signature; - Allocation screening number; Demographic data collection - In case of female patients (collection of information on pregnancy or breastfeeding self-reported by the patient); - Medical and surgical history/current medical conditions; - Prior/concomitant ocular and systemic medications; - Ocular examination of both eyes: o External Ocular Examination; o BCVA test through the ETDRS at 4 m distance; o Symptom Assessment in Dry Eye by SANDE questionnaire; o Evaluation of Intraocular pressure (IOP); o Tear film break-up time with fluorescein (TFBUT)$^{\phi}$; o Ocular surface staining (NEI score) with fluorescein;

	- DEQS questionnaire administration; - Patient eligibility: inclusion/exclusion criteria evaluation; - Assignment of the study and allocation subject clinical investigational number; - Generation of unique subject identifiers code*; - Medical device dispensation; - Beginning of the treatment*. The Investigator will dispense to the patients 5 boxes of medical device each containing 30 single-dose containers of medical device each containing 30 single-dose containers for the following 35±4 days. After completing the baseline evaluation, patients will start the study treatment as instructed through self-instillation of medical device under investigation at home*. Patients will return to the clinical site on day 14±2 from the first instillation of medical device under investigation (Visit 2). **Visit 2 (on the 14th day from the first instillation of medical device under investigation (MD)) (window ± 2 days)***** The following procedures will be performed: - Assessment of treatment compliance; - Current medical conditions; - Concomitant medications; - Evaluation of any reported adverse events /incidents**; - Ocular examination of both eyes: o External Ocular Examination; o BCVA test through the ETDRS at 4 m distance; o Symptom Assessment in Dry Eye by SANDE questionnaire; o Evaluation of Intraocular pressure (IOP); o Tear film break-up time with fluorescein (TFBUT)ϕ; o Ocular surface staining (NEI score) with fluorescein; Patients will return to the clinical site on day 35±4 days from the first instillation of medical device under investigation (Study Termination Visit). **Study Termination Visit (on the 35th day from the first instillation of medical device under investigation) (window ± 4 days)** The final visit is defined as the visit performed on 35 ± 4 days after the first instillation of medical device under investigation°. **In case of premature study discontinuation, patients will undergo an Early Termination Visit (ETV)'.** The following procedures will be performed: - Assessment of treatment compliance (reconciliation of the amount of used single dose containers and empty/unused medical

	device boxes returned by patients to the centre)§; - Current medical conditions; - Concomitant medications; - Evaluation of any reported adverse events/incidents**; - Ocular examination of both eyes: o External Ocular Examination; o BCVA test through the ETDRS at 4 m distance; o Symptom Assessment in Dry Eye by SANDE questionnaire; o Evaluation of Intraocular pressure (IOP); o Tear film break-up time with fluorescein (TFBUT)ϕ; o Ocular surface staining (NEI score) with fluorescein; - Administration of the VAS scale on the degree of satisfaction to the treatment; - DEQS questionnaire administration; - Evaluation of the safety of the experimental product through the compilation of the IGAS scale; - Restitution of remaining medical devices§. * Start of the treatment (window + 1 day). ** In case of adverse events/incidents all assessments considered necessary by the Investigator will be performed, including the possible suspension of treatment. *** Variable because it is based on the planned visit schedule according to clinical practice for the individual patient Visit 2 (windows of ± 2 days). ° All patients enrolled will be treated for 35± 4 days. The Study Termination Visit is variable because it is based on the planned visit schedule according to clinical practice for the individual patient (window ± 4 days). § Restitution of the amount of used single dose containers and empty/unused medical device boxes returned by patients to the centre. 'In case of premature study discontinuation, patients will undergo an Early Termination Visit (ETV). During the ETV the patients will return the amount of used single dose containers and empty/unused medical device boxes returned by patients to the centre. * The "unique subject identifiers code" consists of the 5-digit screening number (e.g. S1001, S1002,….S1028 etc.), and, if applicable, the 4-digit subject clinical investigational number (e.g. 1001, 1002,…1028 etc.). Specifically, the first subject clinical investigational number was referred to the study site. Allocation screening number and subject clinical investigational number are separated by slashes (e.g. "S1001/1001"). (e.g. "S1001/1001" for site number 1, "S2001/2001" for site number 2). ϕ The TFBUT value will be recorded as the average of 3 measurements.

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FLOW CHART

Study Procedure	Visit 1 (screening- enrolment- start of treatment)*	Visit 2*** (on the 14th day from the first instillation of medical device) (window ± 2 days)	Study termination Visit° (on the 35th day from the first instillation of medical device) (window ± 4 days)
Informed Consent	√		
Allocation screening number	√		
Demographic data collection	√		
Medical and surgical history	√		
Current medical conditions	√	√	√
Prior ocular local and systemic medication	√		
Concomitant ocular local and systemic medication		√	√
External Ocular Examination	√	√	√
BCVA evaluation through ETDRS grade	√	√	√
SANDE questionnaire	√	√	√
Evaluation of Intraocular pressure (IOP)	√	√	√
Ocular surface staining (Fluorescein- NEI score)	√	√	√
Tear Film Break-up Time$^{\phi}$ (TFBUT- Fluorescein)	√	√	√
Administration of DEQS$^{\#\#\#}$ questionnaire	√		√
Inclusion/Exclusion Criteria	√		

Enrolment and allocation subject clinical investigational number	√		
Generation of unique subject identifiers code*	√		
Medical device dispensing*	√		
Check Compliance (check proper treatment with medical device, use of other medication and medical devices)		√	√
Medical device restitution§ '			√
Evaluation of satisfaction to the treatment by VAS scale			√
Evaluation of IGAS			√
Adverse event /Incidents**		√	√

* Start of the treatment (window + 1 day).

** In case of adverse events incidents all assessments considered necessary by the Investigator will be performed, including the possible suspension of treatment.

*** Variable because it is based on the planned visit schedule according to clinical practice for the individual patient Visit 2 (windows of ± 2 days).

° All patients enrolled will be treated for 35 ± 4 days. The Study Termination Visit is variable because it is based on the planned visit schedule according to clinical practice for the individual patient (window ± 4 days).

§ Restitution of the amount of used single dose containers and empty/unused medical device boxes returned by patients to the centre.

'In case of premature study discontinuation, patients will undergo an Early Termination Visit (ETV). During the ETV the patients will return the amount of used single dose containers and empty/unused medical device boxes returned by patients to the centre.

*The "unique subject identifiers code" consists of the 5-digit screening number (e.g. S1001, S1002,….S1028 etc.), and, if applicable, the 4-digit subject clinical investigational number (e.g. 1001, 1002,…1028 etc.). Specifically, the first subject clinical investigational number was referred to the study site.

Allocation screening number and subject clinical investigational number are separated by slashes (e.g. "S1001/1001"). (e.g. "S1001/1001" for site number 1, "S2001/2001" for site number 2).

ϕ The TFBUT value will be recorded as the average of 3 measurements.

###In the event that, for both trial centers, the DEQS questionnaire is not available, the assessment of the quality of the life will be performed by the DEQ-5 questionnaire.

LIST OF ABBREVIATION

BVCA= Changes about Best Corrected Visual Acuity
CA = Competent Authority
CIP = Clinical Intestigational Plan
CRF = Case Report Forms
CRO = Contract Research Organization
EC= Ethic Committee
eCRF = electronic Case Report Form
ETDRS = Early Treatment Diabetic Retinopathy Study
ETV = Early Termination Visit
FPFV = First Patient First Visit
GCP = Good Clinical Practice
HCE = Human Corneal Epithelial
ICF = Informed Consent Form
ICH = International Conference on Harmonization
IMP =Investigational Medicinal Product
IOP= Intraocular Pressure
IS = Investigational Site
LPLV = Last Patient Last Visit
MD = Medical Device
MedDRA= Medical Dictionary for Regulatory Activities
NA = Not Applicable
NEI = National Eye Institute
PI = Principal Investigator
PP = Per Clinical investigation plan Set
PT = Preferred Term
RA = Regulatory Authorities
SAF = Safety Analysis Set
SANDE =Symptom Assessment in Dry Eye
SAP = Statistical Analysis Plan
SOC= System / Organ class
SOPs = Standard Operating Procedures
TFBUT = Tear Film Break-Up Time
TSP = Tamarind seed polysaccharide
VAS = Visual Analog Scale

TABLE OF CONTENTS

1. Investigator and Facility

1.1 Principal Investigator and Clinical Research Facility

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1.2 Statistics

All data collected will be analysed by a statistical expert, who will produce a report.

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1.3 Quality Assurance Unit

The study clinical investigation plan review, inspection of the clinical facilities and methods used as well as verification of data records and eCRFs for accuracy and compliance will be performed by the UNIFARM Research Centre Quality Assurance personnel.

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2. Rational and background

Dry eye disease (DED) is a common ocular condition of the ocular surface and tear film caused by tear deficiency or excessive evaporation, leading to damage to the interpalpebral ocular surface [1] and is associated with subjective symptoms, including ocular discomfort, visual disturbance, dryness, and soreness [1, 2].

The disease has a multifactorial origin and linked to pathological conditions of one of the functional unit portions that includes the tear film, and the ocular surface (cornea, conjunctiva, accessory lacrimal glands, the meibomian glands, the junction epidermal mucus, a master gland and nervous connection systems, secretory ducts and lacrimal sac nose).

The prevalence of DED is high, affecting up to 34% of certain populations, particularly female and elderly (> 65 years old) patients with a significant impact patients' quality of life and a significant burden on society [3].

From a pathophysiological point of view, ocular surface inflammation, hyperosmolarity, and tear film instability have been identified as the main triggers for the development and progression of the disease [1].

The stability of the tear film is influenced by its individual components as lipids, water and mucins [4,5]. The open eye is constantly subjected to desiccating stress through evaporation of the tears; however, it is protected from damage by homeostatic mechanisms that regulate tear secretion and distribution in response to signals from the ocular surface. A failure of these mechanisms leads to a quantitative or qualitative deficiency of tears that typically induces tear film instability, wetting defects and hyperosmolar stress, increased friction and chronic mechanical irritation at the ocular surface and an increased risk of infections.

Moreover, the alteration of the precorneal tear film may rapidly result in gland dysfunctions of the eyelids and conjunctiva and ultimately affect the visual acuity (VA) [6].

The classic tear film model is composed of 3 major components: an outer lipid layer, an aqueous middle layer, and the inner mucus layer. Intrinsic or extrinsic changes to any of these three components of the tear film can affect ocular surface homeostasis and encourage tear film instability and tear hyperosmolarity [3].

As extrinsic changes, one major causative risk factor of semi-acute eye complaints is air pollutants causing chemesthesis (sensory irritation) by trigeminal stimulation of the ocular surface [7].

In general hormonal or autoimmune factors aggravated by pollution, local infection, ultraviolet irradiation, tobacco smoke or allergic or iatrogenic phenomena results in a cascade of cellular reactions, in which apoptosis may be closely linked.

Patients affected by ocular dryness are usually treated with topical lubricants with the aim to replace the tear volume, stabilize the tear film, protect the ocular surface and reduce friction of the eyelids on the cornea while blinking. Commercially available tear lubricants differ in their composition and include cellulose derivatives, lipids, polyvinyl alcohol, hydroxypropyl guar and hyaluronic acid [8].

Sodium hyaluronate is a high-molecular-weight anionic polysaccharide with several mechanical properties useful for lubricating the ocular surface. It is a natural polymer whose physical characteristics confer to solutions important viscoelastic properties. It is high hydrophilic and therefore it can bind high quantities of water in a reversible manner, to increase the tear volume and to hydrate the ocular surface. In addition, sodium hyaluronate has an excellent

pseudoplasticity (i.e. non-newtonian behaviour). This means that an ophthalmic topical device containing sodium hyaluronate is more viscous at rest (i.e. when eyes are open) and more liquid-like when a stress is applied (i.e. during blinking), thus minimizing friction between lids and ocular surface acid [9, 10]. This effect is reversible, so that such solution is able to recover its viscosity.

Solutions containing sodium hyaluronate are able to prolong their ocular surface contact time in respect to other ophthalmic products.

A growing number of studies have been published demonstrating good tolerability of sodium hyaluronate and its ability to improve symptoms of dry eye [11] A recent systematic review [12] examined 18 clinical trials comparing the efficacy of sodium hyaluronate against alternative lubricant preparations in the treatment of dry eye syndrome. Majority of the 18 studies selected for review showed superiority of sodium hyaluronate in improving ocular staining and symptoms. Sodium hyaluronate demonstrated greater improvement of Schirmer's test compared to other preparations ($p < 0.001$).

It is generally recognized that elimination of preservatives such as benzalkonium chloride is important in the long-term safety and tolerability of ocular preparations, especially for individuals with dry eye [13].

In this field, the hydrating and viscoelastic properties of sodium hyaluronate make him the ideal candidate in all conditions of ocular redness and/or ocular dryness due to alterations of the lachrymal film. In addition, sodium hyaluronate retains water and therefore, it is proficient to maintain the ocular surface moisturized and protect it from external agents.

Hyalistil BIO PF (listed in the Medical Device Technical File as HA MONO 0.2% Phosp. Free-) is sterile medical devices (eye drops) for ophthalmic use containing 0.2% sodium hyaluronate. The device is presented in unidose containers phosphate-free. Therefore, HA MONO 0.2% Phosp. Free- is indicated to lubricate and hydrate the ocular surface able to provide a stable coating on the surface of the eye performing a moisturizing and lubricating action on the ocular surface, thus allowing a temporary relief to burning, irritation and all dry-eye related discomfort.

In addition, the device assures the protection of the ocular surface during the healing processes.

Moreover, since the presence of phosphates in ophthalmic formulations may rarely cause, in patients with compromised cornea, corneal deposits or corneal opacities [14, 15], HA MONO 0.2% Phosp. Free- is a specific formulation without phosphates (and alternative buffer systems) mainly indicated in all cases of ocular discomfort and ocular dryness due to (as example) dry eye syndrome, ocular surgery, allergy,

environmental factors (sun exposure, wind, smoke, pollution, conditioned air), excessive use of computer monitors, contact lenses use, aging, Moreover, they protect the ocular surface during the process of wound healing after corneal abrasions.

One-two drops of HA MONO 0.2% Phosp. Free- should be instilled in the conjunctival fornix three to four times daily, unless otherwise indicated.

No safety concerns have been identified and reported on the leaflet with the exception of occasional burning and local irritation upon instillation.

Rolando (2007) [16], published a paper describing the activity of HA MONO 0.2% or Tamarind seed polysaccharide (TSP) in the treatment of dry eye syndrome. This was an open-label, randomized, single-centre clinical study. Thirty patients were randomized to receive three or more applications per day of either TSP 0.5%, TSP 1% or HA MONO 0.2%. over a period of 90 days.

The primary objective of tolerability was assessed by visual analogue scale, scoring of specific symptoms and the incidence of adverse events. Secondary objectives included improvement in stability of the precorneal tear film, subjective symptoms and corneal and conjunctival staining. All 3 treatments had a comparable performance. In this study, higher TSP concentration gave higher benefit for the subjective symptoms.
Monaco (2011) [17], analyzed the effects of HA MONO 0.2% or 0.5% carboxymethylcellulose (Optive) on the ocular surface in 20 patients with glaucoma using antiglaucoma drugs. This was a prospective, comparative, randomized, double-blind study with a crossover design. Each group was administered a 4-week course administered quarter in a day. After a 2-week washout period, the course of treatment was reversed. The primary efficacy criteria consisted of assessing symptoms according to the OSDI;
The secondary efficacy criteria consisted of evaluating tear film confocal microscopy, central corneal thickness, corneal and conjunctival staining. At the end of the study only carboxymethylcellulose induced a significant improvement in OSDI and staining compared to baseline values. No significant differences were observed for other parameters evaluated. Both solutions were well tolerated. No adverse events were reported.
Cagini (2017) [18], compared the stability of the tear film after a single instillation of eye drops containing HA MONO 0.2% or 0.2% sodium hyaluronate crosslinked (ICROSS). Forty subjects were included in this study: 20 healthy volunteers and 20 patients suffering from Sjogren syndrome. The surface regularity index and surface asymmetry index were registered before and 5, 30, and 60 min after instillation of eye drops. In healthy subjects the two compounds had no effect, whereas in patients with Sjogren syndrome topographic indexes improved with both compounds; crosslinked 0.2% sodium hyaluronate had a better performance ($P < 0.05$) for both surface regularity index and surface asymmetry index at 60 min after instillation.
Under these premises, considering that HA-MONO-0.2%-Phosp.free was marketed in Italy with the name “Hyalistil Bio PF”, the objective of the present study is to assess the clinical performance, tolerance, and safety of Hyalistil Bio PF device after 35 ± 4 days of treatment in patients affected by eye *discomfor*t in particular in case of ocular dryness.

3. Aim of the Clinical Investigation

The purpose of this prospective observational study is to assess, during the conventional clinical practice, the performance and safety of the daily instillation of Hyalistil Bio PF device in the treatment of patients affected by eye *discomfor*t in particular in case of ocular dryness.
A subject will be included in the study if:

- has been fully informed by the Investigator about the details of the observational study;
- has signed the Informed Consent Form;
- fulfils all the inclusion criteria and if he has not any exclusion criteria.

The assignment of a subject to medical device treatment is not decided in advance by CIP but is part of current clinical practice. The use of the medical device is separated from the decision to include the subject in the clinical investigation. No additional invasive or burdensome diagnostic or monitoring procedures outside at the normal clinical practice are applied to the subjects. Regarding Tear Film Break-Up Time test with fluorescein (TFBUT), fluorescein staining is an

invasive procedure and is normally used in clinical practice in the assessment of ocular discomfort due to dry eye.
Once a subject's eligibility has been established, the Investigator will provide all the relevant information about this clinical investigation.
The Informed Consent will be reviewed, discussed, dated and signed by both the subject and the Investigator before the beginning of screening procedures. Each subject will be interviewed by the Investigator who will record medical history, previous/concomitant pharmacological treatments, and will conduct a routine medical examination.
No laboratory tests are planned.
The data subject (including personal data and special categories of personal data) will be processed only by the authorised scientific staff, as reported in the staff signature and the delegation log.
The Investigator will assign two progressive numerical codes (screening number) to the screened patient and (subject clinical investigation number) to the enrolled patient.
The Investigator will not assign subjects into intervention groups and will not be made any attempts to collect data beyond those available throughout the course of normal clinical practice.
Only the authorised scientific staff will know any additional information regarding the identifiability of the patient. The Sponsor and/or the delegated staff of CRO will receive and/or known anonymized patient data through the numerical code (unique subject identifiers code) assigned by the Investigator.
The unique subject identifiers code consists of the 5-digit screening number (e.g. S1001, S1002,….S1028 etc.), and, if applicable, the 4-digit subject clinical investigational number (e.g. 1001, 1002, 1028, etc.). Specifically, the first subject clinical investigational number was referred to the study site.
Allocation screening number and subject clinical investigational number are separated by slashes (e.g. “S1001/1001”). (e.g. “S1001/1001” for site number 1, “S2001/2001” for site number 2).
The Investigator will only report the information and data in coded form in the clinical study record, referring exclusively to the unique subject identifiers code assigned to the patient at the time of enrolment.
In the unlikely event that authorized staff of CRO becoming aware of the patient's identity or parts of their identity, the CRO can neither transcribe/ report/ copy nor save this information or even communicate or transmit to the Sponsor.
The Investigator and the Sponsor are autonomous data controllers.

4. Clinical Investigation Design

This clinical investigation is a multicentric prospective observational study investigating the effect of daily instillation in patients suffering from eye *discomfort* in particular in case of ocular dryness under normal clinical practice. Withdrawn subjects will be replaced until reaching a total number of 28 subjects.
The study will run for 4 months from first patient’s enrolment until data evaluation. All patients will be followed from enrolment until study end date, which will occur when, in compliance with the normal clinical practice, the last patient will undergo the Study Termination Visit (35± 4 days from the first instillation of study product).

The duration of the enrolment period is expected to be 3 months.

5. Patient risk and benefit

This is a non-interventional clinical investigation and therefore all medical procedures will be performed per standard of care. The clinical investigation plan itself does not therefore introduce any additional risk for the patient.
This clinical investigation plan will collect routine data at specified time points and will not require additional patient visits or investigations.

6. Inclusion criteria

1. Subjects (male or female) must be ≥ 18 years of age;
2. Subject able to provide Informed Consent, in compliance with the good clinical practice and local laws (thus, subject able to comprehend the full nature and purpose of the study, including possible risks and side events);
3. Subjects with ocular discomfort resulting from mild to moderate dry eye in one or both eyes determined by:
   - Scoring of ocular surface staining with fluorescein using the National Eye Institute (NEI) scale. Total score per single eye range 6-33 summing the score of cornea and conjunctiva. (Considering a normal score of 0-33);
   - Tear film break-up time with fluorescein (TFBUT) ≤ 10 seconds; The TFBUT value will be recorded as the average of 3 measurements;
   - Symptom Assessment in Dry Eye (SANDE) questionnaire ≥ 35.
4. Subject able to be compliant with the requirements of the clinical investigation plan, according to the Investigator;
5. Subject who qualifies for Hyalistil Bio PF treatment according to the approved indication;
6. Subject who in physician's opinion will benefit from this treatment.

7. Exclusion criteria

1. Corneal injuries or abrasions of traumatic origin in the eye of study;
2. Ocular infection or clinically significant inflammation (such as Herpes Simplex infection, corneal virus infection, bacterial, viral or fungal conjunctivitis, tuberculosis and mycosis of the eye, purulent and herpetic blepharitis, stye);
3. Sjögren's syndrome;
4. Stevens-Johnson syndrome;
5. Systemic lupus erythematosus;
6. Pathologies associated with corneal thinning;
7. Taking drugs that may interfere with tear gland secretion (beta -blockers);
8. Patients using any topical therapies such as non-steroidal anti-inflammatory drugs, cortisone, cyclosporine, vasoconstrictor, artificial tears (different than the investigated product) the eye of study;
9. Treatment with topical or systemic corticosteroids in the 4 weeks preceding the study;
10. Known or potential hypersensitivity and/or history of allergic reactions to one of the

components of the topical medical device.

11. Participation in another clinical trial within the previous 30 days;
12. Evidence of severe or uncontrolled systemic disease or any other significant disorders, which in the opinion of the Investigator does not allow the participation in the study or could compromise the results.

8. Subject withdrawal criteria

It will be documented whether or not each subject completed the observational study. If, for a subject, observations are discontinued, the reason will be reported.

- Patients can be withdrawn for the following reasons:

✓ Worsening condition despite treatment;
✓ Voluntary subject withdrawal for any reason and at any time without prejudice;
✓ At the discretion of the Investigator, (e.g. lack of patient compliance, etc.);
✓ If an adverse reaction (including a concomitant illness) develops which in the view of the Investigator is incompatible with the continuation of the study;
✓ Failure to comply with requirements of the clinical investigation plan;
✓ Onset of conditions requiring additional treatments or surgery that could influence the results of the observational study;
✓ Any treatment discontinuation must be recorded on the electronic case report form (eCRF) by the Investigator, who will indicate date and reason(s) for treatment withdrawal.

8.1 Discontinuation procedures

For any patient discontinuing the observational study, the Investigator will:
- Ask the patient to undergo, as far as possible, an Early Termination Visit (ETV) to examine the patient's health conditions. This examination will verify that all values tested at screening have remained within a clinically acceptable range (i.e. not clinically significant changes compared to screening);
- Arrange for alternative medical care of the withdrawn patient, if necessary;
- Report in the eCRF the date and time of administration of the last dose and the date and primary reason of study discontinuation.

9. Screening Failure

Screening Failures are considered “Potential subjects who were screened for the participation in the clinical investigation, but were not enrolled”.
Primary reason for screening failure could be:

- Adverse Event;
- Patient Non-compliance;
- Withdrawn of Consent;
- Inclusion / exclusion criteria not met;
- Other.

10. Primary objective

- 25%increase of Tear film break-up time with fluorescein (TFBUT) at Study Termination Visit *(Day 35 ± 4 of treatment)* compared to Visit 1 *(Day 0 of treatment - baseline).*

11. Secondary objective

- Evaluation of the change in total score (score from 6 to 33, considering a normal score 0-33) resulting from the sum of the corneal staining score (score from 0 to 15) and conjunctival staining score (score from 0 to 18) with fluorescein using the National Eye Institute Scale (NEI) at Visit 2 *(Day 14 ± 2 of treatment)* and at the Study Termination Visit *(Day 35 ± 4 of treatment)* compared to Visit 1 *(Day 0 of treatment - baseline);*
- To evaluate the tear film stability per group as objectified by the tear break up time with fluorescein (TFBUT) at Study Termination Visit *(Day 35 ± 4 of treatment)* compared to Visit 2 (*Day 14 ± 2 of treatment*).
- Changes about Best Corrected Visual Acuity (BCVA) measured by the "*Early Treatment Diabetic Retinopathy Study*" (ETDRS) at Visit 2 (*Day 14 ± 2 of treatment*) and Study Termination Visit *(Day 35 ± 4 of treatment)* compared to Visit 1*(Day 0 of treatment – baseline)*.
- Patient reported outcomes:
  To compare patients reported outcomes (PRO) measures per group, including:
  - Patient's reported symptoms (SANDE) at Visit 2 (*Day 14 ± 2 of treatment*) and Study Termination Visit *(Day 35 ± 4 of treatment)* compared to Visit 1*(Day 0 of treatment – baseline)*.
  - Evaluation of the degree of satisfaction to the treatment reported by patients through the use of the visual analogue scale (VAS)[##], at the Study Termination Visit (*Day 35 ± 4 of treatment*).
  - Assessment of the quality of life (QOL)[###] by "Questionnaire about Eye Symptoms and Daily Life" (DEQS)at Study Termination Visit (Day 35 ± 4 of treatment) compared to Visit 1 (Day 0 of treatment - baseline).

[###]In the event that, for both trial centers, the DEQS questionnaire is not available, the assessment of the quality of the life will be performed by the DEQ-5 questionnaire.

Safety Evaluations

- Evaluation of the safety and the *compliance* of medical device.

12. Clinical Investigation Scheme

Study Procedure	Visit 1 (screening- enrolment- start of treatment)*	Visit 2*** (on the 14th day from the first instillation of medical device) (window ± 2 days)	Study termination Visit° (on the 35th day from the first instillation of medical device) (window ± 4 days)
Informed Consent	√		
Allocation screening number	√		
Demographic data collection	√		
Medical and surgical history	√		
Current medical conditions	√	√	√
Prior ocular local and systemic medication	√		
Concomitant ocular local and systemic medication		√	√
External Ocular Examination	√	√	√
BCVA evaluation through ETDRS grade	√	√	√
SANDE questionnaire	√	√	√
Evaluation of Intraocular pressure (IOP)	√	√	√
Ocular surface staining (Fluorescein- NEI score)	√	√	√
Tear Film Break-up Time$^{\phi}$ (TFBUT- Fluorescein)	√	√	√
Administration of DEQS$^{\#\#\#}$ questionnaire	√		√
Inclusion/Exclusion Criteria	√		

Enrolment and allocation subject clinical investigational number	√		
Generation of unique subject identifiers code*	√		
Medical device dispensing*	√		
Check Compliance (check proper treatment with medical device, use of other medication and medical devices)		√	√
Medical device restitution§ '			√
Evaluation of satisfaction to the treatment by VAS scale			√
Evaluation of IGAS			√
Adverse events /Incidents**		√	√

* Start of the treatment (window + 1 day).

** In case of adverse events/incidents all assessments considered necessary by the Investigator will be performed, including the possible suspension of treatment.

*** Variable because it is based on the planned visit schedule according to clinical practice for the individual patient Visit 2 (windows of ± 2 days).

° All patients enrolled will be treated for 35± 4 days. The Study Termination Visit is variable because it is based on the planned visit schedule according to clinical practice for the individual patient (window ± 4 days).

§ Restitution of the amount of used single dose containers and empty/unused medical device boxes returned by patients to the centre.

'In case of premature study discontinuation, patients will undergo an Early Termination Visit (ETV). During the ETV the patients will return the amount of used single dose containers and empty/unused medical device boxes returned by patients to the centre.

* The "unique subject identifiers code" consists of the 5-digit screening number (e.g. S1001, S1002,….S1028 etc.), and, if applicable, the 4-digit subject clinical investigational number (e.g. 1001, 1002,…1028 etc.). Specifically, the first subject clinical investigational number was referred to the study site.

Allocation screening number and subject clinical investigational number are separated by slashes (e.g. "S1001/1001"). (e.g. "S1001/1001" for site number 1, "S2001/2001" for site number 2).

ϕ The TFBUT value will be recorded as the average of 3 measurements.

In the event that, for both trial centers, the DEQS questionnaire is not available, the assessment of the quality of the life will be performed by the DEQ-5 questionnaire.

13. Ethical considerations and Informed Consent

This prospective observational clinical investigation will be performed in compliance with current GCP, including the archiving of essential documents, in compliance with CIP, Declaration of Helsinki, ISO14155:2020, Regulation (EU) 2016/679 concerning the protection of individuals with regard to the processing of personal data (also GDPR), Regulation (EU) 2017/745 (article 63) and other applicable international and national regulatory requirements.
Before starting the observational study, it is necessary that an appropriate Ethics Committee (EC) approve the CIP and Informed Consent Form (ICF).
This is a non-interventional clinical investigation plan and standards of care have to be followed at all times.
Prior to enrolment, at all patients will be provided the ICF. It is the responsibility of the Investigator to ensure that all potential patients are fully informed about the requirements of the study and that they are made aware of the potential risks and benefits of participating in the study. Written confirmation of informed consent has to be obtained from each patient prior to the performance of any study procedure in the trail. Written informed consent must be documented using the study specific ICF. The Investigator (or study personnel) will retain a copy of the signed ICF document and a copy have to be provided to the patient. The date that the ICF is signed must be recorded in the patient's medical notes.
Once approved by the EC, no changes should be made to these documents without first seeking approval from the relevant EC and the Sponsor.

14. Investigational medical device

The medical devices **Hyalistil Bio PF** is moisturizing and lubricating ophthalmic solution helpful in relieving ocular discomfort, such as dry eye syndrome, eye surgery, allergy, and irritation due to environmental conditions (such as pollution, air conditioning, smoke, wind, chlorine in swimming pools, etc.), prolonged use of electronic devices, use of contact lenses, menopause, aging, chronic use of topical drugs (eg. Ocular hypotonizing agents, eye drops containing preservatives, etc.), taking systemic drugs (eg antihypertensives, antidepressants, antihistamines, hormone replacement therapy, etc.).
Hyalistil Bio PF contains sodium hyaluronate (0.2%), a natural polymer presents in various parts of the human body, whose chemical-physical characteristics give the solution viscoelastic, mucoadhesive and moisturizing properties.
Hyalistil Bio PF coats the surface of the eye ensuring adequate lubrication and reducing the friction caused by eyelid movements, hydrates the ocular surface and ensures protection during the repair processes of the corneal epithelium.
Therefore, **Hyalistil Bio PF** guarantees a feeling of lasting well-being and maximum comfort.
The product does not contain preservatives and phosphates, therefore can also be used by contact lens wearers.

14.1 Description of product

Composition

Components	Amount (100 ml)
Sodium hyaluronate	g 0.200
Trometamol (TRIS)	g 0.242
Sodium chloride	g. 0.600
Hydrochloric acid q.s. to	pH 7.2
Potassium chloride	g 0.250
Purified water q.s. to	ml 100

Indications

Hyalistil Bio PF contains sodium hyaluronate, a natural polymer also presents in structures of the human eye, whose chemical physical characteristics gives the solution hydrating, muco-mimetic and viscoelastic properties.

Hyalistil Bio PF coats the eye surface, guaranteeing its adequate lubrification and reducing the friction caused eyelid movements. It also hydrates the ocular surface and assures protection during the healing processes of corneal epithelium.

Therefore, **Hyalistil Bio PF** combines lasting relief with maximum comfort Characteristics and mechanism of action

Presentation

30 single-dose containers of 0.25 ml

Dosage and administration

The patient will instill 1 drop of **Hyalistil Bio PF** in the conjunctival fornix, 4 times a day, in the affected eye.

Instructions for use

Wash / sanitize your hands. Before use make sure that the single-dose container is intact. Detach the single-dose from the strip. Open by turning the top without pulling. Instill avoiding that the tip of the container comes into contact with the eye or any other surface.

Contraindications
Hypersensitivity to one of the ingredients.

Warnings
There are no known interactions of the product with the administration of drugs for ophthalmic use. However, it is recommended not to administer other drugs or medical devices for ophthalmic use within 10 minutes after administration of **Hyalistil Bio PF.**
It is not necessary to remove contact lenses before administering **Hyalistil Bio PF**
as the product does not alter the physical characteristics, including transparency, of the most common soft and rigid contact lenses.
The use of the product may occasionally cause intolerance (mild burning or irritation).

Undesirable effect
The use of the product may occasionally cause intolerance (mild burning or irritation).

Interactions with other drugs or medical device for ocular use:
There are no known interactions of the product with the administration of drugs for ophthalmic use. However, it is recommended not to administer other drugs or medical devices for ophthalmic use within 10 minutes after administration of **Hyalistil Bio PF** It is not necessary to remove the contact lenses before the administration of **Hyalistil Bio PF** since the product does not alter the physical characteristics, including the transparency, of the most common soft and rigid contact lenses.

Precautions for use and storage
For topical ocular use only.
Keep out of reach of children.
Do not use the device if the single-dose is opened or damaged;
Avoid touching the eye or any surface with the tip of the opened container;
Each single-dose container should be used immediately after opening;
Any residual product should be not used. since all devices do not contain preservative and they may become contaminated, exposing the eye to the risk of infection;
Do not use the devices after the expiry date;
Do not dispose the devices in the environment after use;
The administration to children or people with limited ability must be carried out by a responsible adult;
It is advised not to use other drugs or medical device for ocular use within 10 minutes from the administration of the device
The use of the product may occasionally cause intolerance (mild burning or irritation).
Store at a temperature not exceeding 25 ° C.
Validity for intact packaging: 24 months.

14.2 Description as to how traceability shall be achieved during and after the clinical investigation

The traceability will be achieved during and after observational period by the lot number "CC141" assigned to medical device **Hyalistil Bio PF** will be used in the observational study.

14.3 Details concerning the manufacturer of Medical Device

SIFI S.p.A. – Via Ercole Patti, 36
95025 Aci S. Antonio (CT) – ITALY

14.4 Name or number of the model/type to permit full identification

The name of the model to permit full identification of the medical devices is: **Hyalistil Bio PF.**

15. Investigational medical device storage and handling

The Sponsor will offer, like free samples, individual boxes containing an adequate supply of the investigational product to be instilled during the study to each subject.
The Pharmacist and/or Investigator will be responsible for receipt, proper storage, and usage of experimental product, as well as for the investigational product distribution, collection of used and unused vials and final disposal of the remaining investigational product.
The investigational product must be stored not exceeding 25 °C at the investigational sites, in an appropriate locked room accessible only to the pharmacist, the Investigator, or a duly designated person.
A temperature probe and data logger will accompany the investigational product on shipment. It is essential that the investigational sites verify the temperature excursion during shipment against acceptable storage conditions in order to identify potential stability concerns during shipment. These must be immediately communicated to the Sponsor that will decide upon appropriate actions to be taken. The investigational product will be stored in a locked place, sheltered from light.
PI, or a delegate, will maintain an inventory record of the investigational product received.
At the conclusion of the study, a final medical device accountability will be performed. If any supplies are missing, this will be indicated together with an explanation for the discrepancy.
During the Visit 1 the study personnel will give the patient boxes containing the investigational product.
Patient should bring the investigational product, boxes containing for 35±4 days of treatment, at home and store not exceeding 25 °C.
As previously described, each single-dose container must be used immediately after opening.
Do not touch the end of the open container and do not put it in direct contact with the eye.
Each single-dose container has the amount needed to treat both eyes.
Any residue must not be reused, as **Hyalistil Bio PF** does not contain preservatives and the remaining product could become contaminated, exposing to the risk of eye infections.
Any deviations from the recommended storage conditions should be reported by Pharmacist and/or Investigator to the Sponsor, and the use of the investigational product should be suspended

until they have given authorization for its continued use. The investigational product supplies are to be used only in accordance with this clinical investigation plan.

Investigational Medical Devices Delivery Plan

Box delivery	Boxes to be provider
Visit 1 (0 days)	5 Boxes of 30 single dose containers

16. Investigational medical device accountability

The Pharmacist and/or Investigator will confirm the receipt of the investigational product supply in writing by signing and dating standard of investigational product accountability forms.
At the Study Termination Visit the patients will return the used or unused study boxes to the Investigator.
In case of premature study discontinuation, patients will undergo an Early Termination Visit (ETV). During the ETV the patients will return the amount of used single dose containers and empty/unused medical device boxes returned by patients to the centre. The Pharmacist and/or Investigator will keep a cumulative inventory and dispensing records and will maintain all supplies under adequate security.
An accurate product disposition record will be kept, specifying the date and amount dispensed to each patient.
Inventory record of receipt, use or loss of investigational product will be retained. This inventory record must be available for inspection by the Sponsor and regulatory inspection at any time. Copies of this record will be provided to the Sponsor by the CRO throughout the duration of the study.
Partially used or unused study investigational product boxes will be verified and returned by the Investigator/ Institution to the investigational product manufacturer, at the end of the study.
During the observational study, the Investigator will complete the "investigational product accountability forms". After completion of the clinical investigation, the unused study investigational product will be destroyed after authorization by the Sponsor by an authorized company according to GCP regulations.

17. Other allowed therapies

All medications (including over-the-counter drugs, herbal products, vitamins, and antacids) taken prior to start the study or taken for clinical reason during the observational period, will be recorded in the appropriate Case Report Form reporting generic name, date(s) and time(s) of intake, reason(s) for use, and dosage information.
Medication entries should be specific to product name (if a combination drug product) and spelled correctly. The dose, unit, frequency, route of administration, start date, discontinuation date, and indication should also be recorded. For medications administered only one time, the frequency column may reflect "once."
Concomitant treatments should be taken according to current medical practice. All therapies, topic or systemic, that might interfere with the evaluation of the treatment under investigation are

forbidden. This decision depends on the type of illness and the kind of drug to be used. If the subject must take drugs, the PI will decide whether the subject can continue to participate in the study.
Patients that:

- using any topical therapies such as non-steroidal anti-inflammatory drugs, cortisone, cyclosporine, vasoconstrictor, artificial tears (different than the investigated product) the eye of study;
- must be treated with therapies forbidden (treatment with topical or systemic corticosteroids in the 4 weeks preceding the study);
- treatment with drugs that may interfere with tear gland secretion (beta -blockers);
- must be treated with therapies, that in the opinion of the Investigator does not allow the participation in the study or could compromise the results;
- will be excluded from the prosecution of the study, but their data until that moment will be used in the statistical analysis.

18. Procedures

Study visits and procedures

In this observational study each enrolled patient will be evaluated during 3 scheduled visits.
Maximum observation period will be 35 ± 4 days. A written informed consent will be obtained before any study assessment or procedure.
The first patient first visit (FPFV) is defined as the 1st visit to the clinical site by the 1st screened patient. The "Last Patient Last Visit" (LPLV) is defined as the last visit to the clinical site by the last patient (i.e., the last visit foreseen by the study clinical investigation plan), independently of whether the patient completed or withdrew from the study.
All enrolled subjects will be treated for 35 ± 4 days and followed for a maximum of 39 days. They will be evaluated during 3 scheduled visits according to clinical practice for the individual patient: at baseline (Visit 1), at 14 ± 2 days from the first instillation of medical device under investigation (Visit 2) and at 35 days from the first instillation of medical device under investigation (Study Termination Visit) with window of ± 4 days.

Visit 1 (screening-enrolment)

The following procedures will be performed:

- Explanation to the patient of study aims, procedures and possible risks of the observational study;
- Informed consent signature;
- Allocation screening number;
- Demographic data collection;
- In case of female patients (collection of information on pregnancy or breastfeeding self-reported by the patient);
- Medical and surgical history/current medical conditions;
- Prior/concomitant ocular and systemic medications;

- Ocular examination of both eyes:
  - External Ocular Examination;
  - BCVA test through the ETDRS at 4 m distance;
  - Symptom Assessment in Dry Eye by SANDE questionnaire;
  - Evaluation of Intraocular pressure (IOP);
  - Tear film break-up time with fluorescein (TFBUT)$^{\phi}$;
  - Ocular surface staining (NEI score) with fluorescein;
- DEQS questionnaire administration;
- Patient eligibility: inclusion/exclusion criteria evaluation;
- Assignment of the study and allocation subject clinical investigational number;
- Generation of unique subject identifiers code*;
- Medical device dispensation;
- Beginning of the treatment*.

The Investigator will dispense to the patients 5 boxes of medical device each containing 30 single-dose containers of medical device each containing 30 single-dose containers for the following 35±4 days. After completing the baseline evaluation, patients will start the study treatment as instructed through self-instillation of medical device under investigation at home*.
Patients will return to the clinical site on day 14±2 from the first instillation of medical device under investigation (Visit 2).

Visit 2 (on the 14th day from the first instillation of medical device under investigation (MD)) (window ± 2 days)***

The following procedures will be performed:
- Assessment of treatment compliance;
- Current medical conditions;
- Concomitant medications;
- Evaluation of any reported adverse events/incidents**;
- Ocular examination of both eyes:
  - External Ocular Examination;
  - BCVA test through the ETDRS at 4 m distance;
  - Symptom Assessment in Dry Eye by SANDE questionnaire;
  - Evaluation of Intraocular pressure (IOP);
  - Tear film break-up time with fluorescein (TFBUT)$^{\phi}$;
  - Ocular surface staining (NEI score) with fluorescein;

Patients will return to the clinical site on day 35±4 days from the first instillation of medical device under investigation (Study Termination Visit).

Study Termination Visit (on the 35th day from the first instillation of medical device under investigation) (window ± 4 days)

The final visit is defined as the visit performed on 35 ± 4 days after the first instillation of medical

device under investigation°.

In case of premature study discontinuation, patients will undergo an Early Termination Visit (ETV)'.

The following procedures will be performed:

- Assessment of treatment compliance (reconciliation of the amount of used single dose containers and empty/unused medical device boxes returned by patients to the centre)§;
- Current medical conditions;
- Concomitant medications;
- Evaluation of any reported adverse events/incidents**;
- Ocular examination of both eyes:
  - External Ocular Examination;
  - BCVA test through the ETDRS at 4 m distance;
  - Symptom Assessment in Dry Eye by SANDE questionnaire;
  - Evaluation of Intraocular pressure (IOP);
  - Tear film break-up time with fluorescein (TFBUT)ϕ;
  - Ocular surface staining (NEI score) with fluorescein;
- Administration of the VAS scale on the degree of satisfaction to the treatment;
- DEQS questionnaire administration;
- Evaluation of the safety of the experimental product through the compilation of the IGAS scale;
- Restitution of remaining medical devices§.

* Start of the treatment (window + 1 day).

** In case of adverse events/incidents all assessments considered necessary by the Investigator will be performed, including the possible suspension of treatment.

*** Variable because it is based on the planned visit schedule according to clinical practice for the individual patient Visit 2 (windows of ± 2 days).

° All patients enrolled will be treated for 35 days. The Study Termination Visit is variable because it is based on the planned visit schedule according to clinical practice for the individual patient (window ± 4 days).

§ Restitution of the amount of used single dose containers and empty/unused medical device boxes returned by patients to the centre.

'In case of premature study discontinuation, patients will undergo an Early Termination Visit (ETV). During the ETV the patients will return the amount of used single dose containers and empty/unused medical device boxes returned by patients to the centre.

* The "unique subject identifiers code" consists of the 5-digit screening number (e.g. S1001, S1002,….S1028 etc.), and, if applicable, the 4-digit subject clinical investigational number (e.g. 1001, 1002,…1028 etc.). Specifically, the first subject clinical investigational number was referred to the study site.

Allocation screening number and subject clinical investigational number are separated by slashes (e.g. "S1001/1001"). (e.g. "S1001/1001" for site number 1, "S2001/2001" for site number 2).

ϕ The TFBUT value will be recorded as the average of 3 measurements.

19. Primary and secondary outcomes^

***Primary Endpoints*[#]**

- *Clinical performance:*

-25 % increase from Visit 1 (*Day 0 of treatment - baseline*) to Study Termination Visit (*Day 35 ± 4 of treatment*) of tear film break-up time with fluorescein (TFBUT)f. [Time frame: Visit 1 and Study Termination Visit].

Secondary Endpoints

- Reduction from baseline (*Visit 1- Day 0 of treatment*) in the corneal and conjunctival fluorescein staining score using the NEI scale.

Fluorescein staining of the ocular surface is assessed as the total sum of the corneal and conjunctival subregions.

- Corneal Fluorescein Staining (NEI score, sum of 5 subregions, maximum score 15. Each subregion will have a maximum score of 3.
(0=no staining, 3 = maximum staining).
- Conjunctival Fluorescein Staining (NEI score, sum of 6 subregions, maximum score 18. Each subregion will have a maximum score of 3.
(0=no staining, 3 = maximum staining).
[Time frame: Visit 1, Visit 2 and Study Termination Visit].

- Tear film break-up time with fluorescein (TFBUT)[ϕ]. [Time frame: Visit 2 and Study Termination Visit].
- BCVA is measured using the standard Early Treatment Diabetic Retinopathy Study (ETDRS) acuity chart and reported in ETDRS letters. [Time frame: Visit 1, Visit 2 and Study Termination Visit];
- *Patient reported outcomes:*

- Change in intensity and frequency of dry eye symptoms assessed by completing the questionnaire SANDE. [Time frame: Visit 1, Visit 2 and Study Termination Visit].
- [##]Evaluation of the degree of satisfaction to the treatment reported by patients through the use of the visual analogue scale (VAS)[##], ranging from 0 to 100 mm, at Study Termination Visit (*Day 35 ± 4 of treatment*). [Time frame: Study Termination Visit].

Patients will be asked to rate their degree of satisfaction to the treatment and answer the following questions:

- "I feel satisfied using this treatment? ",
- "With this treatment, I have a feeling of freshness?",
- "With this treatment, I have a feeling of relief?",
- "This treatment contributed to reduce my pain due to eye dryness?",
- "This treatment is comfortable? ",

Patients will be asked to place a vertical mark on a horizontal line, from 0 to100 mm, to indicate the degree of satisfaction to the treatment, where:

✓ 0-10 mm=none

✓ 11-30 mm=very mild
✓ 31-50 mm=mild
✓ 51-70 mm=moderate
✓ 71-90 mm=strong
✓ 91-100 mm=very strong.

- Assessment of the quality of life (QOL) by "Questionnaire about Eye Symptoms and Daily Life" (DEQS) at Study Termination Visit (Day 35 ± 4 of treatment) compared to Visit 1 (Day 0 of treatment - baseline). [Time frame: Visit 1 and Study Termination Visit].

###In the event that, for both trial centers, the DEQS questionnaire is not available, the assessment of the quality of the life will be performed by the DEQ-5 questionnaire.

Evaluation of safety parameters

Evaluation of safety of daily instillation of ophthalmic solution during all the study period through:

- Evaluation of safety of daily instillation of ophthalmic solution during all the study period through:
- Investigator Global Assessment of Safety (IGAS): using the 4-point scale:1= very good safety, 2 =good safety, 3 = moderate safety and 4 = poor safety. IGAS will be evaluated at the Study Termination Visit. [Time Frame: Study Termination Visit];
- Evaluation of reported adverse events/incidents. [Time Frame: During the treatment period].
- Evaluation of intraocular pressure (IOP) [Time frame: Visit 1, Visit 2 and Study Termination Visit];
- Evaluation of *compliance* through verification of correct instillation of the medical device, counting of single dose containers and boxes. [Time Frame: Visit 2 and Study Termination Visit].

^According to clinical practice, the Investigator will perform all clinical evaluations on both eyes of the individual patient.

The statistical analysis will be performed considering right eye as the "study eye."

#The primary end point will be evaluated on the ITT population and in PP population.

20. Equipment to assess clinical investigation variables and primary and secondary outcomes

Ophthalmological Evaluations

Ocular evaluations will be performed on both eyes. The assessment will be performed at Baseline (Visit 1), Visit 2, and at the Study Termination Visit. The ophthalmological assessment will include:

External Ocular Examination

External Ocular Examination assesses the motility of the extraocular muscles and the appearance and function of the eyelids before instillation of any dilating or anesthetic eye drops.

Best Corrected Visual Acuity

Best Corrected Visual Acuity BCVA was measured using the standard Early Treatment Diabetic Retinopathy Study (ETDRS) acuity chart lighting, and procedures and reported in
Best-corrected visual acuity is measured at all trial visits using standard charts, (chart 1 is used for testing the visual acuity of the RIGHT eye; Chart 2 for testing the LEFT eye).
Patients should not be allowed to see any of the charts before the examination**.**
A distance of 4 meters is required between the patient's eyes and the visual acuity chart. With the box light off, not more than 15 foot-candles of light (161.4 Lux) should fall on the center of the chart. To measure the amount of light, the room is set up for visual acuity testing, but with the box light off. The light meter is placed at the fourth line from the top of the chart, with its back against the chart and the reading is taken. If more than one line available for testing visual acuity, the visual acuity of an individual patient should be measured in the same line at each visit, if possible. If different lines are used to test visual acuity, they must each meet the same standards.

Ocular surface staining (NEI score - fluorescein)

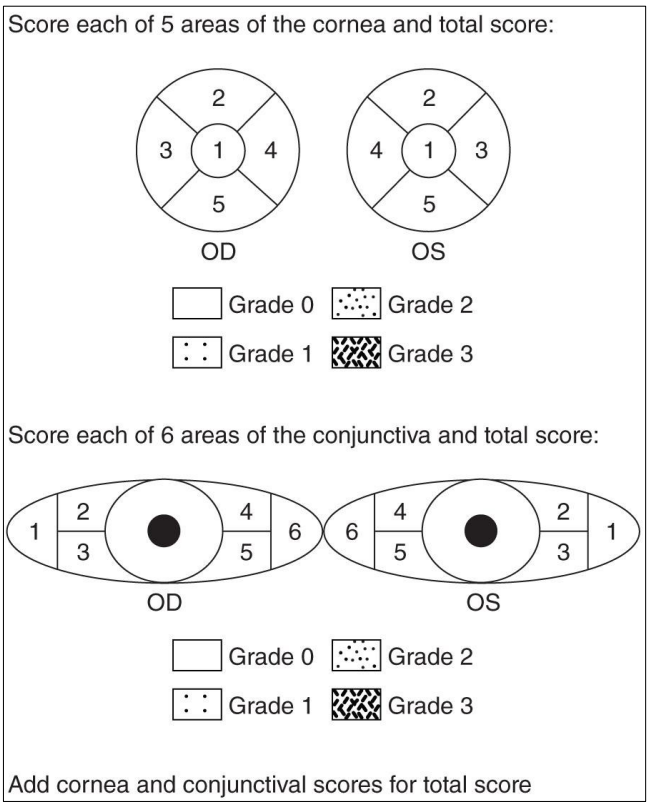


Figure from: NEI Industry grading system, American Academy of Ophthalmology: https://www.aao.org/

As a grading scale for the corneal and conjunctiva damage, the NEI/Industry Workshop guidelines will be used. The cornea is divided into five sectors (central, superior, inferior, nasal and temporal), each of which is scored on a scale of 0–3, with a maximal score of 15. Both nasally and temporally, the conjunctiva is divided into a superior paralimbal area, an inferior paralimbal area and a peripheral area with a grading scale of 0–3 and with a maximal total score of 18 for the nasal and temporal conjunctiva.
For a better reading it is also essential not to use an intense illumination beam, which may reduce the contrast and lead to an underestimation of grading.

Intraocular pressure (IOP) assessment
Intraocular pressure (IOP) measurement was performed using a Goldmann applanation tonometer after topical anesthesia with unpreserved 0.4% oxybuprocaine hydrochloride.

Symptom Assessment in Dry Eye (SANDE):
The Symptom Assessment in Dry Eye (SANDE) questionnaire is a short questionnaire to evaluate both the intensity and frequency of dry eye using a 100 mm VAS [19]. The patient's symptoms of ocular dryness and/or irritation will be quantified on the scale based on two questions assessing both severity and frequency of symptoms. A VAS is a measurement instrument that tries to measure a characteristic or attitude that is believed to range across a continuum of values and cannot easily be directly measured. For example, the amount of irritation that a patient feels ranges across a continuum from none to an extreme amount of irritation. From the patient's perspective this spectrum appears continuous (i.e. their irritation does not take discrete jumps, as a categorization of none, mild, moderate and severe would suggest). It was to capture this idea of an underlying continuum that the VAS was devised.
For the assessment, the patients mark on the 100 mm VAS line the point that they feel represents their perception of their current state. The VAS score is determined by measuring in millimeters from the left-hand end of the line to the point that the patient marks. The SANDE scores will be then evaluated for the 2 questions severity (dryness and irritation with a range of 0-100 for each question) and frequency (dry and irritated with a range of 0-100 for each question). To simplify the procedure the patient will be informed that to provided anchors for the question measuring the frequency of symptoms, the extreme left of the 100 mm line indicated "rarely" and the extreme right indicated "all of the time". Similarly, for the question that measured the severity of symptoms, the words "very mild" and "very severe" were placed at the left and right ends of the 100 mm line, respectively.

SANDE Questionnaire

PLEASE COMPLETE THE FOLLOWING QUESTIONS REGARDING THE FREQUENCY AND SEVERITY OF YOUR DRY EYE SYMPTOMS.

1. Frequency of symptoms:
Please place an 'X' on the line to indicate how often, on average, your eyes feel **dry and/or irritated:**

Rarely ——— **All the time**
0 **100**

2. Severity of symptoms:
Please place an 'X' on the line to indicate how severe, on average, you feel your symptoms of **dryness and/or irritation**:

Very Mild ——— **Very Severe**
0 **100**

Tear Film Break-up Time (TFBUT)

Patients with a Tear Film Break-up Time TFBUT test ≤ 10 in the study eye (study eye) at the screening visit are eligible for enrollment.

TFBUT will be measured by determining the time to tear break-up. The TFBUT will be performed after instillation of 5 µl of 2% preservative-free sodium fluorescein solution into the inferior conjunctival cul-de-sac of each eye. The patient will be instructed to blink several times to thoroughly mix the fluorescein with the tear film. To achieve maximum fluorescence, the examiner should wait approximately 30 seconds after instillation before evaluating TFBUT. With the aid of a slit lamp at 10X magnification using cobalt blue illumination, the examiner will monitor the integrity of the tear film, noting the time it takes to form lacunae (clear spaces in the tear film) from the time that the eye is opened after the last blink. This measurement will be performed within 10 seconds maximum. The TFBUT will be measured twice during the first minute after the instillation of the fluorescein. The TFBUT value will be the average of 3 measurements.

Relevant TFBUT findings will be entered in the eCRF.

Safety of Medical Devices

Considering the observational nature of the study, a surveillance program to collect adverse events /incidents/potential incidents was not planned.

Adverse events /incidents/potentially incidents should be reported in the same way as spontaneous post-marketing reports.

The safety will be evaluated by the collection of eventual presence of adverse events /incidents/potentially incidents that happened from the ICF signature until the end of the 35 ± 4 days study. All adverse effect/incidents/potentially incidents (independently from the seriousness) experienced during the study, spontaneously reported by the patient, or elicited as

a result of general questioning by the site staff, will be recorded in the source documents (Clinical Record) and they will be reported on the eCRF.
Furthermore, the Safety of medical device will be assessed using the Investigator Global Assessment of Safety (IGAS): using the 4-point scale:1= very good safety, 2 =good safety, 3 = moderate safety and 4 = poor safety. IGAS will be evaluated during the Study Termination Visit. [Time Frame: Study Termination Visit].

Visual Analogue Scales

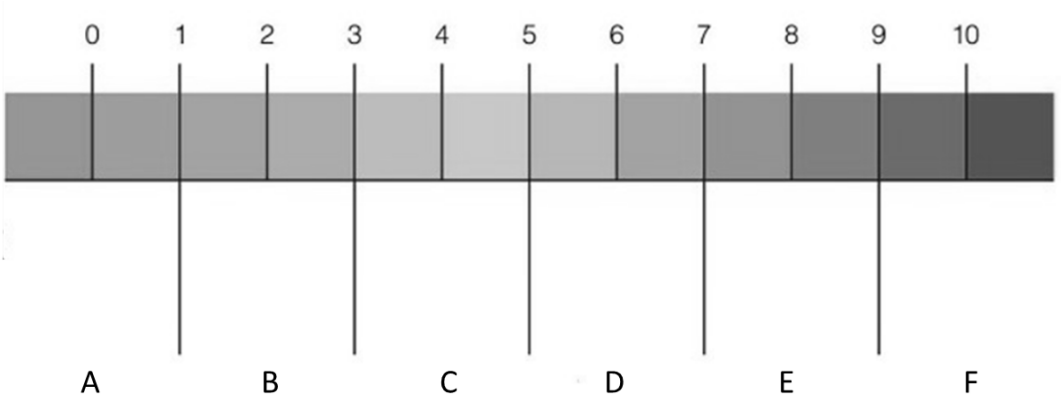
Patients will be asked to rate their degree of satisfaction to the treatment and answer the following questions:

- "I feel satisfied using this treatment? ",
- "With this treatment, I have a feeling of freshness?",
- "With this treatment, I have a feeling of relief?",
- "This treatment contributed to reduce my pain due to eye dryness?",
- "This treatment is comfortable? ",

Patients will be asked to place a vertical mark on a horizontal line, from 0 to100 mm, to indicate the degree of satisfaction to the treatment, where:

✓ 0-10 mm=no
✓ 11-30 mm=very mild
✓ 31-50 mm=mild
✓ 51-70 mm=moderate
✓ 71-90 mm=strong
✓ 91-100 mm=very strong.

Evaluation of the degree of satisfaction to the treatment reported by patients through the use of the visual analogue scale (VAS) ranging from 0 to 100 mm, at Study Termination Visit [Time frame: Study Termination Visit].



100 mm = 10 cm 1= 10 cm
A= 0-10 cm=no
B =11-30 cm=very mild
C= 31-50 cm=mild
D= 51-70 cm=moderate
E = 71-90 cm=strong
E= 91-100 cm=very strong

Dry Eye-Related Quality-of-Life Score **(DEQS)**

The DEQS questionnaire consists of 15 items to assess symptoms and their effect on daily living throughout the previous week.

During the study will be asks to patients to rate the frequency and severity of various ocular symptoms on a scale of 0 to 4 from “not at all” to “always” and “not at all” to “very much.”

The first six questions focus on ocular symptoms, while the other nine focus on how the patient's daily life has been affected. It questions patients on things like light sensitivity and difficulty using screens, whether their work is being impacted and whether they are feeling depressed as a result of their symptoms. A quality-of-life score ranging from 0 to 100 will be then calculated with the cutoff value for DED being 15 points.

In the event that, for both trial centers, the DEQS questionnaire is not available, the assessment of the quality of the life will be performed by the DEQ-5 questionnaire.

Dry Eye Questionnaire (DEQ-5) measures symptom severity over the last month. The test contains only five questions, making it one of the quickest to complete and grade. During the study will be asks patients to rate the severity of eye discomfort, dryness and wateriness each from 0 to 4, with 0 indicating "never" and 4 indicating "constantly." The patient will be asked about the intensity of the symptom, with a score of 0 meaning it is not intense at all, and a score of 5 meaning it is very intense. The total score is a number between 0 and 22.

All measurements will be taken by a trained optometrist and all instruments will be calibrated immediately prior to the study.

21. Statements of compliance

22.1 Declaration of Helsinki

The CRO/Sponsor and the Investigator ensure that all procedures described in this clinical investigation plan, data evaluation and documentation will be produced strictly according to normal clinical practice and the current version of Declaration of Helsinki.

22.2 Ethic Committee and Regulatory Authority Approvals and Any Additional Requirements

The clinical investigation plan and essential documents (ICF, etc..) will be approved by the Ethics Committees (ECs) and notified to the Ministry of Health. The approval of the clinical investigation will be obtained before the beginning of the study.
The study can begin only after full approval by the EC. Medical device will not be sent to Investigator/s before the EC.

22.3 Informed Consent

Before enrollment in the study, the Investigator will:

1. Explain, in a clear and detailed manner, the scope, the procedures and the possible consequences of the clinical investigation. The information will be given in both oral and written form.
2. Obtain signed informed consent from the potential subject before performing any of the procedures included in the observational study.
3. Assign screening number;
4. Determine subject eligibility;
5. Assignment of the study and allocation of subject clinical investigational number;
6. Generation of unique subject identifiers code that consist of the 5-digit screening number (e.g. S1001, S1002,…S10028 etc.), and, if applicable, the 4-digit subject clinical investigational number (e.g. 1001, 1002,…1028 etc.). Specifically, the first subject study number was referred to the study site.
   Allocation screening number and subject clinical investigational number are separated by slashes (e.g. “S101/1001”).

If a subject withdraws from participation in the observational study, his/her unique subject identifiers code can’t be reused.
Before being enrolled in the clinical investigation, the patients must have expressed their consent to participate by provide a written consent form. The information sheet and informed consent form will be prepared in the local language by the CRO and must be approved by the EC. It will include all the elements required by law according to the ICH-GCP recommendations.
In addition to the standard requirements that Investigators are obliged to observe in providing information, the following points must be covered:

- a description of the aims of the observational study and how it will be organised, in

accordance with the normal clinical practices;
- the type of treatment;
- any potential adverse events attributable to the clinical investigation treatment;
- the freedom to ask for further information at any time;
- the right of subjects to withdraw from the clinical investigation at any time without giving reasons and without compromising their further course of medical treatment;
- adequate time to answer possible questions.

Adequate time and opportunity to satisfy questions will be given to the patients and the time will be recorded.
The Investigator will be supplied with an adequate number of blank informed consent forms to be used. The forms will be signed and dated by both the Investigator and the subjects.
A copy of the signed form will be given to the patient.
To ensure medical confidentiality and data protection, the signed informed consent forms will be stored in the Investigator's study file according to the regulatory requirements.
The Investigator will allow inspection/audit of the forms by authorized representatives of the Sponsor, EC members and Competent Authorities (CA). He will confirm, by signing and dating the forms, that informed consent has been obtained. In case any new information regarding the IDs will be available during the study the subject will be contacted by the Investigator and in the case a new version of informed consent, will be prepared, submitted to the EC and after approval proposed to the subject for signature.
The Informed Consent will incorporate (or, in some cases, be accompanied by a separate document incorporating) the wording that complies with relevant data protection and privacy legislation (Regulation (EU) 2016/679 concerning the protection of individuals with regard to the processing of personal data, also GDPR).

22.4 Amendments and EC Additional Requirements

In order to obtain interpretable results, neither the Investigator nor the Sponsor will alter the clinical investigation conditions agreed upon and set out in this clinical investigation plan. Amendments should be made by mutual agreement between the Investigator and the Sponsor. Any amendment to this study clinical investigation plan must be set out in writing, giving the reasons, being signed by all concerned parties and will be sent to the EC. All amendments will be sent to the ECs.
Any amendments that may lead to increased risks and/or inconvenience to the subject must first be approved by the EC, if appropriate, and such approval in writing addressed to the Sponsor.
The amendment becomes then part of the clinical investigation plan.
Copies of all documents must be stored also by the Investigator/s. Any additional EC requirements will be followed as appropriate.

22.5 Insurance Policy

As the study is non-interventional, there is no need for insurance policies in addition to those already provided for normal clinical practice.

22. Subject Compliance

The Subjects' compliance will be monitored by study staff during visits and evaluated by the Investigator. Any subject found not to follow pre-study directions or to be non-compliant, will be withdrawn from the study. Details of the reason for removal of subjects will be recorded and reported.

23. Statistic

Sample size estimate

A previous study report baseline level of TFBUT of 6.23±1.75 in patients with moderate dry eye disease. After 30 days of treatment with tear eyedrops containing hyaluronic acid 0.3%, TFBUT was 8.10 ± 2.06 s [20]. To detect a 25% of increase of TFBUT at Study Termination Visit compared to the baseline (Visit 1) (mean difference 1.56, s.d. 1,91), considering an alpha = 0.01 (two-tailed) and a power of 1 – ß = 0.80, 23 subjects are required (Wilcoxon signed-rank test). Taking into account an expected dropout of 20%, it is expected to enroll a total of 28 subjects.

Analysis set

All patients enrolled will be included in the safety population.
All patients enrolled having a primary post-baseline performance assessment will be included in the PP population and ITT population.
If the primary performance measurement at Visit 2 is missing, it will be imputed with the score at baseline. If the primary performance measurement at Study Termination Visit is missing, it will be imputed with the score at Visit 2, if it is not missing, or otherwise with the score at baseline.
All patients of the ITT population who did not have any major clinical investigation plan deviation will be included in the PP population.
The analysis of safety endpoints will be performed in the safety population. Analysis of performance endpoints will be performed on the ITT population. The analysis of primary endpoint will be performed on the PP population but this later will be repeated in the ITT population.

Statistical Methods

Descriptive analysis will be performed by using absolute rate, percentage and frequency tables for qualitative variables.
Normally distributed continuous data will be reported as minimum, maximum, mean and standard deviation, whereas non- normally distributed data as medians and IQR.
Categorical variables will be analyzed by using Yates-corrected chi-square test or Fisher's exact test when appropriate. The McNemar's test will be used to analyze paired nominal data. Differences in endpoints between baseline and different timepoints will be assessed using paired

t-test for normally distributed data, whereas the Wilcoxon signed-rank test will be used for non-normally distributed data.
Data distribution will be evaluated through the Kolmogorov-Smirnov test.
A p-value <0.05 will be considered statistically significant.
Analysis will be performed using SAS version 9.4 for Windows (SAS Institute Inc., Cary, NC, USA).

Primary endpoint analysis

- 25 % increase from Visit 1 (*Day 0 of treatment - baseline*) to Study Termination Visit (*Day 35 ± 4 of treatment*) of tear film break-up time with fluorescein (TFBUT)$^{\phi}$ will be reported as minimum, maximum, mean, standard deviation or median and IQR. According to the data distribution, paired t-test or Wilcoxon signed-rank test will be used to test the statistical significance of the change from baseline to each time points. [Time frame: Visit 1, and Study Termination Visit].

Secondary endpoint analysis

- Changes in NEI score from baseline will be summarized as minimum, maximum, mean, standard deviation or median and IQR. Paired t-test or Wilcoxon signed-rank test will be used to test the statistical significance of the change from baseline to each timepoints. [Time frame: Visit 1, Visit 2 and Study Termination Visit].

- Change in the SANDE scores will be summarized as minimum, maximum, mean, standard deviation or median and IQR. Paired t-test or Wilcoxon signed-rank test will be used to test the statistical significance. Details of changes (i.e., difference in SANDE scores between baseline and end of treatment) will be summarized as well. [Time frame: Visit 1, Visit 2 and Study Termination Visit].

- VAS will be summarized reporting the number of patients and related percentage in each category. [Time frame: Study Termination Visit].

- Changes in TFBUT from baseline will be reported as minimum, maximum, mean standard deviation or median and IQR. Paired t-test or Wilcoxon signed-rank will be used to test the statistical significance of the change from baseline to each timepoints. [Time frame: Visit 2, and Study Termination Visit].

- Change in the BVCA scores will be summarized as minimum, maximum, mean, standard deviation or median and IQR. Paired t-test or Wilcoxon signed-rank test will be used to test the statistical significance. Details of changes (i.e., difference in BVCA scores between baseline and end of treatment) will be summarized as well. [Time frame: Visit 1, Visit 2 and Study Termination Visit].

- IGAS will be summarized reporting the number of patients and related percentage in each category. [Time frame: Study Termination Visit].

- Incidents will be coded using the last updated version of the MedDRA dictionary to give a preferred term (PT) and a system/organ class term (SOC) for each event. The number of patients who experienced at least one incident, study product-related incidents, serious incidents, and the number of patients withdrawn due to an incident will be summarized. The frequency of incidents will be presented overall, by SOC and PT, and additional grouping by severity and relationship to the observed treatment will be performed. [Time Frame: During the treatment period].

- Changes from baseline in IOP will be summarized as minimum, maximum, mean standard deviation or 18 median and IQR. Paired t-test or Wilcoxon signed-rank will be used to test the statistical significance of the change from baseline to each timepoints. [Time frame: Visit 1, Visit 2 and Study Termination Visit].

- Changes from baseline in DEQS score will be summarized as minimum, maximum, mean, standard deviation or median and IQR. Paired t-test or Wilcoxon signed-rank test will be used to test the statistical significance of the change from baseline to the study termination. [Time frame: Visit 1 and Study Termination Visit].

24. Data collection – eCRFs

An electronic data capture system will be used for this study. An electronic CRF (eCRF) is designed to collect all the data required by the clinical investigation plan and collected by the investigator for each patient.
The Investigator must ensure that the clinical data required by the clinical investigation plan, collected during the study, are carefully reported, in the eCRFs. He must also check that the data reported in the eCRFs correspond to those in the patients' source documents.
Details of eCRF completion and correction will be explained to the Investigator. If the Investigator authorizes other persons to make entries in the eCRF, the names, positions, signatures, and initials of these persons must be supplied to the Sponsor.
The Investigator or the designated personnel of their team agrees to complete all documents provided by the Sponsor at each patient's visit and complete the eCRF within 2 days from the patient's visit.
Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.
The monitors must make certain that all data are completed on the eCRF and are according to source.
Before the lock of the database by the Data Management Department, the Investigator must attest

by entering his/her user's name and password:

- the authenticity of the data collected in the eCRF;
- the coherence between the data in the eCRF and those in the source documents.

The Investigator must keep source documents for each subject in the clinical investigation. All information on eCRFs must be traceable to these source documents, which are generally stored in the subject's medical file. The source documents will contain all demographic and medical information, as well as the original signed informed consent form.
The monitor, in accordance with the Sponsor's requirements, should ensure that the trial is properly conducted and documented.
The monitor checks the accuracy and completeness of eCRF entries, source documents, and other records related to the trial and notifies (monitoring report) the Sponsor of deviations from the clinical investigation plan, SOPs, GCP, and applicable regulatory requirements, taking appropriate action to prevent recurrence of identified deviations.
The monitor should submit a written report to the Sponsor after each trial- site visit or clinical investigation-related communication (monitoring report).
Reports should include a summary of what the monitor reviewed and the monitor's statements concerning the significant findings/facts, deviations and deficiencies, conclusions, actions taken or to be taken and/or actions recommended to secure compliance. The review and follow-up of the monitoring report with the Sponsor should be documented by the Sponsor's designated representative.
Any change or correction to eCRF should be dated, initialed, and explained (if necessary) and should not obscure the original entry.
The systems are designed to permit data changes in such a way that the data changes are documented and that there is no deletion of entered data (i.e. maintain an audit trail, data trail, edit trail).

25.1 Unique subject identifier

All the patients who sign the informed consent form for this study will be coded with "unique subject identifiers".
The unique subject identifier consists of the 5-digit screening number (e.g. S1001, S1002,…S10028 etc.), and, if applicable, the 4-digit subject clinical investigational number (e.g. 1001, 1002, etc.). Specifically, the first subject study number was referred to the study site.
Allocation screening number and subject clinical investigational number are separated by slashes (e.g. "S101/1001" for site number 1, "S201/2001" for site number 2, "S301/3001" for site number 3).

Study Sites	**Screening Number**	**Subject Clinical Investigational Number**
SITE Number 1	S1001, S1002, S1003,	1001, 1002, 1003, 1004,

P.O. San Marco, Azienda Ospedaliero Universitaria Policlinico "G. Rodolico - San Marco" Oculistica, Viale Carlo Azeglio Ciampi, 95121 Catania (Italy) Tel: +39 0953781213 Email:davidescollo@hotmail.com	S1004, S1010, S1028etc.	etc.
SITE Number 2 U.O. di Oculistica, Presidio Belmonte, Azienda Ospedaliero Universitaria Policlinico "Paolo Giaccone" Via Rampolla Mariano Cardinale, 10B, 90142 Palermo (Italy) Tel: +39 091 6553903 Email: enzabonfiglio@gmail.com	S2001, S2002, S2003, S2004, S2028 etc.	2001, 2002, 2003, 2004, etc.

25.2 Coding dictionaries

Medical/surgical history and underlying diseases, physical examination abnormalities and AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA™).
Prior and concomitant medications will be coded using the WHO Drug Dictionary Enhanced (WHODDE). Version of coding dictionaries will be stated in the SAP and the study report. Study Monitoring, Quality Control and Quality Assurance.

26 Monitoring, Data and Quality Management

26.1 Clinical monitoring and identification of source data

Monitoring is the act of overseeing the progress of a clinical trial and of ensuring that it is conducted, recorded and reported in accordance with the clinical investigation plan, SOPs, Good Clinical Practice (GCP), and the applicable regulatory requirement(s).
UNIFARM will designate qualified person(s) to maintain a close liaison with the Investigators and study staff to ensure the study is being conducted with respect to GCP and according to the approved clinical investigation plan and subsequent amendment(s).
This liaison will consist of documented visits and follow-up letters, and/or telephone call or Email communication, in accordance with monitoring plan, before the start of study and at regular intervals during the study to allow for the periodic reviews of the progress of the study.
UNIFARM is responsible to verify that all incidents have been transmitted to the Sponsor and to the Competent Authority and the Ethic Committee if deemed necessary.

The CRA will explain the clinical investigation plan and study related procedures to all study staff, including the Investigator. If new collaborators are included during the clinical investigation, additional training sessions will be organized by the Investigator and/or the CRA.
The main responsibilities of the monitor CRA are to verify Investigators' adherence to the clinical investigation plan and that informed consent is obtained and recorded for all patients prior to implementation of any study procedure.
The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the eCRF are known to the Sponsor and participating study-site personnel and are accessible for verification by the CRA.
The CRA will contact and visit the Investigator at regular intervals during the study. He will compare the eCRFs with medical records and other relevant documentation through direct access, during the on-site monitoring visits. He verifies the completeness, consistency and accuracy of the data being recorded in the CRF by the Investigator.
The Sponsor expects that, during any monitoring visits, the relevant participating study-site personnel will be available, the source documentation will be accessible, and a suitable environment will be provided for review of observational study-related documents.
The confidentiality of the subjects' identities shall be well protected consistent with local and national regulations when the source documents are subject to direct access.
The monitor will provide the Investigator with all the study materials before the study starts and, during the course of the study, will check at least the following:
• Full compliance with ICH-GCP, ISO 14155:2020, EU Regulation 745/2017, GDPR, the clinical investigation plan and applicable regulatory requirements;
• Subject recruitment;
• Subject compliance;
• Medical Device accountability;
• Completeness and accuracy of the eCRF data and their consistency with the source documents (appropriate source data verification will be conducted on all eCRFs);
• Verification of the facilities;
• Investigator's Site File.
The monitor will ensure completeness of eCRFs on an ongoing basis.
Study monitor will have access to all records (including source documents when applicable) necessary to ensure the integrity of the recorded data. During monitoring visits, the Investigators and authorized staff will assist the monitor to check the eCRFs and other documents pertinent to the project.
As part of the supervision of the study progress, other Sponsor personnel or the CRO may, on request, accompany the CRA on visits to the study site. The Investigator and his/her collaborators commit to cooperate with the CRA to resolve any problems, corrections or possible misunderstandings concerning the findings detected in the course of these monitoring visits.

26.2 Source Documents

Source documents are defined as original documents, data and records. This may include hospital

records, clinical and office charts, laboratory data/information, pharmacy dispensing and other records. Data collected during this study must be recorded on the appropriate source documents.

26.3 Audit

The audit is an independent verification, separate from the monitoring activity, of the activities and the documents to ensure that the activities pertinent to the study were duly carried out and that they were recorded, analyzed and transferred in compliance with the clinical investigation plan, GCP, relevant SOPs and with applicable legislation.

26.4 Inspections

Authorized representatives of national and foreign Competent Authorities may perform an official review of the study site, including, study site and source data verification. The purpose of inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the clinical investigation plan, GCP, ICH guidelines, and any applicable regulatory requirements. The Investigator/Institution will contact immediately the Sponsor if contacted by a regulatory agency about an inspection at the study site.

25. Safety Data Evaluation

All safety data from medical examinations will be documented in the eCRFs, summarized by descriptive statistics and the results will be included in the clinical investigation Report.

26. Adherence to Clinical Investigation Plan

Except for an emergency situation in which proper care or medical treatment is required for protection, safety and well-being of the subjects, the clinical investigation will be conducted as described in the approved clinical investigation plan in accordance with the normal clinical practice. Any deviation from the normal clinical practice will be recorded and explained.

27. Clinical Investigation Termination

- Regular Termination of the clinical investigation

The observational study completion date is the date on which the last subject is examined or receives an intervention for the purpose of final data collection, if the observation period for this subject is completed according to the clinical investigation plan.

- Premature Termination of the clinical investigation

The Sponsor and Investigator reserve the right to termination the observational study at any time. Reasons for discontinuation must be documented appropriately.

Upon completion of the clinical investigation, the patients will be ensured medical care at the same clinical site, according to the times and procedures required by normal clinical practice.

28. Financing of the Clinical Investigation

The financial aspects of this clinical investigation are described in detail in the contract between Sponsor and CRO and between CRO and clinical site involved in this study.

29. Clinical Investigation Report

The final Clinical Investigation Report will be written by the CRO and then approved by "Sponsor", and the Principal Investigator. It will be written in compliance with the ISO: 14155:2020, EU Regulation 745/2017 (Annex XV) and GDPR for both content and format.

30. Publication policy

The Sponsor agrees that the clinical results (including negative and inconclusive as well as positive results) can be made publicly available by the Investigator publishing in peer reviewed journals; presenting results at scientific congresses; and posting information and results on internet-based public registers and databases.

In any case, study results will be communicated in full to the competent Authorities by the submission of a complete Clinical Study Report.

As the Sponsor agrees that the study results can be published by the Investigator(s), the Investigator agrees to submit any manuscript (abstract, publication, paper etc.) to the Sponsor before any public disclosure.

This will be done in order to ensure that clinical trial results are reported in an objective, accurate and balanced manner. The Sponsor reviews proposed manuscripts prior to submission within a reasonable period of time (30-90 business days in relation with the complexity of the work).

The Investigator(s) will also be provided by the Sponsor with the clinical study report and the results of any additional analysis, tables, figures etc. undertaken for the purposes of the article, in order to take responsibility for the content of the publication(s).

On an exceptional basis, the Sponsor may temporarily delay registration of certain data elements (e.g. compound, name, outcome, measures etc.) to seek necessary intellectual property protection. This is because early disclosure of such a data could, in some circumstances, prevent or negatively impact patentability.

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