STATISTICAL ANALYSIS PLAN

Investigation CCI - Sponsor code LUB0116MD

A 2 week, randomized, double-masked, controlled, parallel group study to evaluate tolerability, safety, permanence on the ocular surface and efficacy of two concentrations of Lubricin (20 and 50 µg/mL) eye drops versus sodium hyaluronate (HA) 0.18% eye drops (Vismed®) in patients with ocular discomfort following refractive surgery

A 2 week randomized (1:1:1), controlled, double-masked, parallel group, pre-market study

TEST 1 product:	Lubricin 20 µg/mL eye drops
TEST 2 product:	Lubricin 50 µg/mL eye drops
CONTROL product:	Vismed® - Sodium hyaluronate (HA) 0.18% eye drops
Manufacturer/Sponsor:	Dompé farmaceutici s.p.a., Via San Martino, 12 2012 Milano, Italy Operative Offices: Via Santa Lucia 6 20122 Milano, Italy
Clinical Site Name:	Università La Sapienza- Policlinico Umberto I Dipartimento "Organi di Senso" Viale del Policlinico 155 00161 Rome (RM) - Italy Tel.: PPD Fax: PPD
Site Co-ordinating Investigator:	PPD, MD
Study Phase:	Pre-market
Version and date:	Final version 1.0, 22SEP2017

This study is conducted in compliance with the protocol, attachments VIII and X of legislation decree 24 February 1997, n.46 and s.m.i., Declaration of Helsinki, Good Clinical Practice (GCP) as set forth in the International Conference on Harmonization (ICH) guidelines on GCP (ICH E6), ISO14155-2012 and applicable local regulatory requirements

This document comprises 32 pages plus appendices

VERSIONS' HISTORY

Version	Date of Issue	Reason for change
Draft version 0.1	PPD 2017	PPD issued the first draft
Draft version 0.2	PPD 2017	PPD fixed a typo error ("in" instead of "is", see section 7.2 PK parameters) PPD added the listing of device administration at site (see section 8.8 Investigational device administration at site) PPD added the change from pre-dose at Day 1 and Day 15±2 in the listing of Lubricin concentrations (see section 8.10 Lubricin concentrations)
Final version 1.0	PPD 2017	PPD issued the final version 1.0 after Sponsor's approval

APPROVAL AND ACKNOWLEDGEMENT

MANUFACTURER/SPONSOR
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Biometry Unit Representative (for approval and acknowledgement)

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Date

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Signature

Medical Writing Team Representative (for approval and acknowledgement)

PPD PPD

PPD

Date

PPD

Signature

Coordination (for acknowledgement only)

PPD

PPD

Date

PPD

Signature

INVESTIGATION SCHEDULE

	Visit 1	Visit 2	Visit 3/ETV
Evaluation	**Day 1 - Baseline**	**Day 15 ± 2**	**Final Follow-up Visit Day 22 ± 2**
Informed Consent	**X**		
Inclusion/Exclusion Criteria	**X**		
Pregnancy test for females	**X**	**X**	
Eligibility/Enrolment	**X**		
Demography	**X**		
Medical/Surgical history (ocular &systemic)	**X**		
Review of current medical conditions (ocular & systemic)		**X**	**X**
Prior and concomitant medications (ocular & systemic)	**X**	**X**	**X**
External Ocular Examination	**X**	**X**	**X**
VAS ocular tolerability assessment	**X****	**X****	**X**
SANDE questionnaire	**X**	**X**	**X**
Best corrected distance visual acuity	**X**	**X**	**X**
Slit lamp examination (SLE)	**X**	**X**	**X**
Central corneal sensitivity by Cochet-Bonnet aesthesiometry	**X**	**X**	**X**
Tear collection for Lubricin dosing	**X*****	**X*****	**X******
Tear Film Break Up Time (TFBUT)	**X**	**X**	**X**
Corneal fluorescein staining (Oxford score)	**X**	**X**	**X**
Schirmer's test (ST) I without anesthesia	**X**	**X**	**X**
Intraocular pressure (IOP)	**X**	**X**	**X**
Self-administration at home	**X***	**X***	
First Administration at study site	**X**	**X**	
Compound Dispensing	**X**		
Adverse events	**X**	**X**	**X**

*Self-administration at home during the study period
** pre-dose, 15 minutes post–dose, 30 minutes post–dose
*** pre-dose – in both eyes, 15 minutes post–dose in the right eye, 30 minutes post–dose in the left eye
**** both eyes

TABLES OF CONTENTS

ABBREVIATIONS

AE	Adverse Event
ADE	Adverse Device Event
ANOVA	Analysis of variance
BCDVA	Best Corrected Distance Visual Acuity
BLQL	Below Lower Quantitation Limit
CPL	Clinical Project Leader
CDISC	Clinical Data Interchange Standards Consortium
CI	Clinical Investigation
CIMF	Clinical Investigation Master File
CIP	Clinical Investigation Plan
CIR	Clinical Investigation Report
CRF	Case Report Forms
CRO	Contract Research Organization
DD	Device Deficiencies
DEWS	Dry Eye WorkShop
DMC	Data Monitoring Committee
EC	Ethics Committee
ETDRS	Early Treatment Diabetic Retinopathy Study
FAS	Full Analysis Set
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HA	sodium hyaluronate
IB	Investigation Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ID	Investigational Device
IF	Investigator File
IOP	Intraocular Pressure
IEC	Independent Ethics Committee
IS	Investigational Site
IV	Intravenous
MedDRA	Medical Dictionary of Regulatory Activities
MD	Medical Device
mL	milliliters
mmHg	millimeters of Mercury
µg	Micrograms
NA	Not Applicable
NGF	Nerve Growth Factor
NK	Neurotrophic Keratitis
PI	Principal Investigator(s)
PT	Preferred Term
PTAE	Pre-Treatment Adverse Event
PR	Pulse Rate
TID	Three times per day
RA	Regulatory Authorities
SAE	Serious Adverse Events
SADE	Serious Device Events

SANDE	Symptom Assessment iN Dry Eye
SAP	Statistical Analysis Plan
SLE	Slit Lamp Examination
s.m.i.	following change and /or additions
SOC	System Organ Class
SOP	Standard Operating Procedure
SDTM	Study Data Tabulation Model
ST	Schirmer's test
TEAE	Treatment-Emergent Adverse Event
TF	Technical File
TFBUT	Tear Film Break Up Time
VA	Visual Acuity
VAS	Visual Analogical Scale
WHODDE	World Health Organization Drug Dictionary Enhanced

1 INTRODUCTION

Statistical analysis will be performed by the CRO Biometry Unit. The endpoints and methods of analysis specified in this SAP are consistent with ICH E6 (R2), E9 and ISO14155-2012 guidelines (2, 3, 4). The SAP has been compiled by the CRO Biometry Unit on the basis of the final version 2.0 of the clinical investigational plan (5), reviewed by the Sponsor and finalized before the database lock and the treatment unblinding.

1.1 Changes with respect to the investigational plan

The following changes were introduced into the SAP with respect to the clinical investigational plan (5):

- The tears collection for Lubricin dosing was insert as PK endpoint;
- The device deficiencies, external ocular examination, review of ocular medical condition and review of systemic medical condition were reported as safety and tolerability parameters.

These changes do not affect the principal features stated in the investigational plan and thus do not have to be documented in a protocol amendment.

2 INVESTIGATON OBJECTIVES

The primary objective of the investigation is to assess the tolerability and safety of Lubricin (20 and 50 µg/mL) eye drops solution administered over 2 weeks in patients with ocular discomfort following refractive surgery.

Secondary objectives of the investigation are aimed at studying efficacy, including ocular surface permanence, of Lubricin (20 and 50 µg/mL) eye drops solution administered over 2 weeks versus sodium hyaluronate (HA) 0.18% eye drops solution in patients with ocular discomfort following refractive surgery.

2.1 Primary endpoints

1) Tolerability using a Visual analogue scale (VAS) for dryness, foreign body sensation, burning/stinging, itching, pain, stick feeling, blurred vision and photophobia;
2) Treatment-emergent adverse events (TEAEs), assessed throughout the study.

2.2 Secondary endpoints

1) Ocular surface vital staining with Fluorescein (Oxford scale);
2) Schirmer-I test (without anaesthesia);
3) Permanence of Lubricin on the Ocular Surface Tear film break-up time (TBUT);
4) Best corrected distance visual acuity (BCVA);
5) SANDE questionnaire scores – discomfort improvement entity;
6) SANDE questionnaire scores – discomfort improvement speed;
7) Signs evaluated by Slit lamp examination (SLE) (blepharitis, eyelid hyoeremia/oedema, lashes, conjunctiva hyperemia);
8) Intraocular pressure (IOP);

9) Corneal sensitivity by Cochet-Bonnet aesthesiometry.

2.3 PK endpoint

1) Tears collection for Lubricin dosing.

3 INVESTIGATIONAL PLAN

3.1 Overall investigation design

A 2 week randomized (1:1:1), controlled, double-masked, parallel group, pre-market study.

Patients with ocular discomfort following refractive surgery procedure (within 6 months from enrollment into the investigation) will be evaluated at baseline (Day 1), at Week 2 (day 15±2 days) and at Week 3 follow-up visit (day 22±2, or early exit).

3.2 Discussion of design

The investigation has been designed in agreement with the ICH E6 (R1) and ISO14155-2012 guidelines (1, 4).

The aim of the investigation is to evaluate tolerability, safety permanence on the ocular surface and efficacy of two concentrations of Lubricin (20 and 50 µg/mL) eye drops versus sodium hyaluronate (HA) 0.18% eye drops (Vismed®) in patients with ocular discomfort following refractive surgery.

Enrolled subjects will be randomized at inclusion, 1:1:1 ratio to receive Lubricin 20 µg/ml eye drops solution or Lubricin 50 µg/ml eye drops solution or sodium hyaluronate (HA) 0.18% eye drops solution. Upon randomization and until the end of the treatment period, subjects will be required to not use topical ophthalmic medications except of the study treatments.
On the Day 1 Visit (Baseline visit) subjects will be dispensed of the study medication and instructed to self-administer at home three times a day approximately every 6 hours for 2 weeks.

Subjects will be evaluated after 2 weeks of treatment and 1 week after end of treatment.
The subjects will be involved in the study for a maximum of 25 days from screening visit to last follow-up visit.

4 INVESTIGATION POPULATION

4.1 Target population

Subjects suffering of ocular discomfort following refractive surgery.

4.2 Investigation population

All subjects suffering of ocular discomfort following refractive surgery who are already under treatment at the Policlinico Umberto I or who are diagnosed as being affected of ocular discomfort following refractive surgery during a visit at the Policlinico Umberto I.

4.3 Method of sampling

The investigation sample is the sample of subjects chosen from the investigation population that are included into the investigation.

A simple random sampling strategy was used in this investigation.

All eligible subjects willing to participate to the investigation were included.

4.4 Inclusion criteria

1. Patients 18 years of age or older.
2. Patients undergone ocular refractive surgery within 6 months from Day 1 Visit.
3. Patients with ocular discomfort defined as SANDE score ≥ 30 at baseline.
4. Average VAS score (dryness, foreign body sensation, burning/stinging, itching, pain, stick feeling, blurred vision and photophobia) ≥ 25 mm;
5. Best corrected distance visual acuity (BCDVA) score ≥ 0.1 decimal units in both eyes at the time of study enrolment.
6. Only patients who satisfy all Informed Consent requirements may be included in the study. The patient and/or his/her legal representative must read, sign and date the Informed Consent document before any study-related procedures are performed. The Informed Consent form signed by patients and/or legal representative must have been approved by the IEC for the current study.

4.5 Exclusion criteria

1. Patients with a severe Dry Eye condition (intensity level 4 according to the Report of the International Dry Eye Workshop -DEWS, 2007)
2. Best corrected distance visual acuity (BCDVA) score of < 0.1 decimal units in either eye at the time of study enrolment
3. Evidence of an active ocular infection in either eye
4. History or presence of ocular surface disorders other than ocular discomfort in either eye

5. Use of any ocular topical medication other than the study medications for the treatment of ocular diseases including artificial tears during the study period
6. Use of topical cyclosporine, topical corticosteroids or any other topical medication for the treatment of dry eye in either eye within 30 days of study enrolment
7. History of any ocular surgery (excluding laser or refractive surgical procedures) in either eye within 30 days before study enrolment. Ocular surgery will not be allowed during the study treatment period and elective ocular surgery procedures should not be planned during the duration of the follow-up period
8. Known hypersensitivity to one of the components of the study or procedural medications
9. Participation in another clinical study at the same time as the present study or within 90 days of screening/baseline visit
10. History of drug, medication or alcohol abuse or addiction.
11. Females of childbearing potential (those who are not surgically sterilized or post-menopausal for at least 1 year) are excluded from participation in the study if they meet any one of the following conditions:
    a. are currently pregnant or,
    b. have a positive result on the urine pregnancy test at the Screening/Baseline Visit or,
    c. intend to become pregnant during the study treatment period or,
    d. are breast-feeding or, not willing to use highly effective birth control measures, such as: Hormonal contraceptives – oral, implanted, transdermal, or injected and/or mechanical barrier methods – spermicide in conjunction with a barrier such as a condom or diaphragm or IUD during the entire course of and 30 days after the study treatment periods.

4.5.1 Not allowed treatments

Use of topical cyclosporine, topical corticosteroids or any other topical medication for the treatment of dry eye in either eye is not allowed during the treatment period.

During the study follow-up period (from day 15±2 to day 22±2), any ocular topical treatments are allowed, including other artificial tears/lubricants as prescribed by the treating physician.

Hormonal contraceptives for females will be allowed.

5 INVESTIGATION SCHEDULE

The schedule of the investigation is summarised at page 5.

5.1 Investigation visits and procedures

A written informed consent will be obtained before any study assessment or procedure. Maximum study duration will be 25 days, including all study visits.

Each study subject will undergo to 3 visits.

The first subject first visit (FSFV) is defined as the 1st visit performed at the clinical site by the 1st enrolled subject. The last subject last visit (LSLV) is defined as the last visit performed at the clinical site (or the telephonic follow-up, if applicable) by the last subject, i.e. the last visit foreseen by the study protocol, independently of the fact that the subject is a completer or a withdrawn subject.

The following phases, visits and procedures will be performed:

- **Interventional phase**
  - Visit 1 - Day 1
  - Visit 2 - Day 15 ± 2
- **Follow-up phase**
  - Visit 3 - Final Visit follow-up - (Day 22±2) /ETV (Early Termination Visit) in case of early discontinuation, discontinued subjects will undergo an early termination visit (ETV).

The procedures/assessments details are summarized hereafter.

	Day	Procedures/Assessments
Visit 1 – Day 1 – Baseline – Pre-Dose	*Day 1 pre-dose*	➢ Explanation to the patient of study aims, procedures and possible risks ➢ Informed consent signature ➢ Screening number (as S01, S02, etc.) ➢ Demographic data ➢ Medical and surgical history/current medical conditions ➢ Prior/concomitant medications ➢ External Ocular Examination ➢ AE monitoring ➢ Ocular examination in both eyes: 1. Assessment of ocular tolerability (Visual analogue scale, VAS: dryness, foreign body sensation, burning/stinging, itching, pain, stick feeling, blurred vision and photophobia) 2. Assessment by SANDE questionnaire 3. Assessment of best corrected distance visual acuity 4. Slit lamp examination (SLE) to assess the Eyelid - Meibomian glands, Eyelid Erythema, Eyelid – Oedema, Lashes, Conjunctiva Erythema, Lens, Iris, Anterior Chamber, Corneal transparency, Corneal neovascularization 5. Assessment of central corneal sensitivity by Cochet-Bonnet aesthesiometry 6. Tear collection for Lubricin dosing (both eyes) using tear wash technique with 40 µL of sterile saline solution **At least 10 minutes break** 7. Tear Film Break Up Time (TFBUT) 8. Corneal fluorescein staining (Oxford score) **At least 15 minutes break** 9. Schirmer's test (ST) I (without anaesthesia) 10. Intraocular pressure (IOP) ➢ Pregnancy test for female patients. ➢ Inclusion/exclusion criteria evaluation ➢ Patient eligibility confirmation and randomization number assignment ➢ At the completion of the pre-dose assessments, the investigator will administer the study product (morning dose) into the eligible eye(s)
Baseline - Visit 1	*Day 1 15 minutes post-dose*	The following procedures will be performed: ➢ Assessment of ocular tolerability (Visual analogue scale, VAS: dryness, foreign body sensation, burning/stinging, itching, pain, stick feeling, blurred vision and photophobia) ➢ Tear collection for Lubricin dosing in the right eye

	Day	Procedures/Assessments
Baseline - Visit 1	*Day 1 30 minutes post-dose*	The following procedures will be performed: ➢ Assessment of ocular tolerability (Visual analogue scale, VAS: dryness, foreign body sensation, burning/stinging, itching, pain, stick feeling, blurred vision and photophobia) ➢ Tear collection for Lubricin dosing in the left eye ➢ The Investigator will deliver to the patients the investigational medical device for the treatment period (15±2 days)
At home	*From Day 1 to Week 2± 2 Days Visit*	➢ Self-administration at home of the Investigational medical device TID approximately every 6h into the eligible eye(s)
Visit 2	*Week 2 ± 2 Days (prior the morning dose)*	The following procedures will be performed before receiving the first eye drop treatment of the day: ➢ Review of current medical conditions (ocular and systemic: including External Ocular Examination) ➢ AE and concomitant medications ➢ Ocular examination in both eyes, consisting of the assessments detailed for the baseline visit (points 1-10), in the same order and with the same intervals ➢ Urine Pregnancy test (only female) ➢ At the completion of the pre-dose assessments, the investigator will administer the study product (morning dose) into the eligible eye(s)
Visit 2	*Week 2 ± 2 Days 15 minutes post-dose*	The following procedures will be performed: ➢ Assessment of ocular tolerability (Visual analogue scale, VAS: dryness, foreign body sensation, burning/stinging, itching, pain, stick feeling, blurred vision and photophobia) ➢ Tear collection for Lubricin dosing in the right eye
Visit 2	*Week 2 ± 2 Days 30 minutes post-dose*	The following procedures will be performed: ➢ Assessment of ocular tolerability (Visual analogue scale, VAS: dryness, foreign body sensation, burning/stinging, itching, pain, stick feeling, blurred vision and photophobia) ➢ Tear collection for Lubricin dosing in the left eye

	Day	Procedures/Assessments
Visit 3/ETV Final Follow-Up Visit	*Days 22±2*	The final visit is defined as the visit performed 7±2 days after last administration (day 21 ± 2). In case of study discontinuation subjects will undergo an early termination visit. The following procedures will be performed: ➢ Review of current medical conditions (ocular and systemic: including External Ocular Examination) ➢ AE and concomitant medications ➢ Ocular examination in both eyes, consisting of the assessments detailed for the baseline visit (points 1-10), in the same order and with the same intervals ➢ Patient discharge

6 INVESTIGATION SUBJECT IDENTIFICATION METHOD AND TREATMENT ASSIGNMENT METHOD

6.1 Unique subject identifier

All the subjects who sign the informed consent form for the present study will be coded with "unique subject identifiers" when data are extracted from the study database into the domains of the CDISC SDTM model. The unique subject identifier for study site consists of the Sponsor study code (i.e. LUB0116MD), the 3-digit screening number (e.g. S01, S02, etc.) and, if applicable, the 2-digit subject study number (e.g. 01, 02, etc.).
Study code, screening number and subject study number are separated by slashes (e.g. "LUB0116MD/S01/01").

6.2 Subject identifier for the investigation

The last 5 digits of the unique subject identifier (randomized subjects), corresponding to the subject screening and subject study numbers separated by a slash, or the last 3 digits of the unique subject identifier (not randomized subjects), corresponding to the subject screening number, will appear as subject identifier in the individual listings and figures of the clinical study report and will be used to identify the subjects in in-text tables or wording (if applicable).

6.3 Randomization

The randomization list was computer-generated by the CRO Biometry Unit, using the PLAN procedure of the SAS® version 9.3 (TS1M1) (6) according to the following specification:

- Randomization scheme: 1:1:1
- Block size: 6
- Stratification factors: None

The randomization list will be attached to the final clinical study report.

6.4 Treatment allocation

Subjects were assigned to the treatment arms according to the randomization list.

Randomization numbers were given to the subjects on study day 1 and were used to assign the treatment arms according to the randomization list.

6.5 Blinding

This is a double blind study. Both the investigators and the subjects are not aware of the treatment administered.

Individual envelopes identified with the subject assignment number were prepared. Each one includes the treatment arm assigned to each subject. These envelopes, duly closed and sealed, are included into the Investigator File (IF).

During the study the integrity of the envelopes was regularly checked by CRO Monitor during the visits at site. At the end of the study all the individual envelopes must be returned to Dompé.

The sealed envelope can only be opened in case of emergency, when knowledge of the treatment arm is essential for treating the subject.

Only in case of real need the Investigator may open the sealed envelope. In case of opening, all the details (date, time, reasons….) must be specified into the CRF and the Sponsor must be informed within 24 hours.

If an envelope is opened before the end of the trial, the subject is excluded from the trial.

Copies of the individual envelopes identified with the subject assignment number were also sent to the Dompé Materiovigilance Responsible. Dompé Materiovigilance unblinds a patient treatment arm only for safety reason and documents envelope opening; unblinding is not mandatory before regulatory submission of a reportable SAE/Investigational Medical Device Deficiency.

7 INVESTIGATION EVALUATION PARAMETERS

7.1 Efficacy parameters

7.1.1 Primary efficacy parameters

- Visual analogue scale (VAS) for dryness, foreign body sensation, burning/stinging, itching, pain, stick feeling, blurred vision and photophobia.

7.1.2 Secondary efficacy parameters

- Ocular surface vital staining with Fluorescein (Oxford scale);
- Schirmer-I test (without anaesthesia);
- Permanence of Lubricin on the Ocular Surface Tear film break-up time (TBUT);
- Best corrected distance visual acuity (BCVA);
- SANDE questionnaire scores – discomfort improvement entity;
- SANDE questionnaire scores – discomfort improvement speed;
- Signs evaluated by Slit lamp examination (SLE) (blepharitis, eyelid hyoeremia/oedema, lashes, conjunctiva hyperemia);
- Intraocular pressure (IOP);
- Corneal sensitivity by Cochet-Bonnet aesthesiometry.

7.2 PK parameters

- Lubricin concentrations in tears.

7.3 Safety and tolerability parameters

- Treatment-emergent adverse events (TEAEs), assessed throughout the study;
- Device deficiencies;
- External ocular examination;
- Review of ocular medical condition;
- Review of systemic medical condition.

8 STATISTICAL METHODS

The data documented in this study will be listed by subject, treatment, eye (enrolled eye(s), left and right) and evaluation time point, i.e. Baseline or Day 1 (Visit 1), Day 15 ±2 (Visit 2) and follow-up (Visit 3).

Appropriate descriptive statistics will be used according to the nature of the variable.

Not available data will be evaluated as "missing values". The statistical analysis will be performed using SAS® version 9.3 (TS1M1) for Windows® (6) or higher.

8.1 Tables, listings and figures layout

Tables, listings and figures will be provided according to the following settings:

- Background: White
- Foreground: Black
- Font face: Times
- Font style: Roman
- Font size: 10 pt
- Font weight: Medium (data, footers and notes), Bold (titles and headers)
- Font width: Normal
- Layout: Landscape
- Top Margin: 1.3 cm
- Bottom Margin: 1.3 cm
- Left Margin: 2 cm
- Right Margin: 2 cm
- Test labels: Lubricin 20 µg/mL, Lubricin 50 µg/mL
- Control label: Vismed® 0.18%
- Date format: ddMMMyyyy
- Means, standard deviations, percent coefficient of variations, medians, lower confidence limits and upper confidence limits will be rounded to one digit more than the original data
- Minima and maxima will keep the same number of decimal digits as the source values
- p-values will be rounded to the fourth decimal digit and will be flagged by an asterisk (*) in case of statistical significance (i.e. p-value < 0.05 or, in case of centre by treatment interaction, p-value < 0.10)
- p-values lower than 0.0001 will be reported as "<.0001 *".

The data and results of Lubricin 20 µg/mL and Lubricin 50 µg/mL will be presented before the data and results of Vismed® 0.18% in all listings and tables.

8.2 Analysis sets

8.2.1 Definitions

A subject will be defined as screened after the signature of the informed consent, regardless of the completion of all the screening procedures.

A subject will be defined as randomized in the study if he/she is assigned to a treatment arm (Lubricin 20 µg/mL and Lubricin 50 µg/mL or Vismed® 0.18%).

- ***Enrolled Set***: all enrolled subjects. This analysis set will be used for demographic, baseline and background characteristics
- ***Full Analysis Set (FAS)***: all randomized subjects, who receive at least one dose of the ID. This analysis set will be used for the efficacy analysis
- ***Safety Set***: all subjects who receive at least one dose of the investigational medicinal product(s). This analysis set will be used for tolerability and safety analyses

Each subject will be coded by the CRO Biometry Unit as valid or not valid for the Enrolled set, FAS and Safety set. Subjects will be evaluated according to the treatment dose they actually received.

8.2.2 Reasons for exclusion from the Full Analysis Set

According to ICH E9 guideline (3), the reasons for exclusion of subjects from the Full Analysis Set are the following:

- failure to take at least one dose of the IDs;
- lack of any efficacy data post enrolment;
- failure to satisfy inclusion/exclusion criteria (eligibility violations). Subjects who fail to satisfy an inclusion/exclusion criterion may be excluded from the analysis without the possibility of introducing bias only under the following circumstances:
  - the entry criterion was measured prior to randomization;
  - the detection of the relevant eligibility violations can be made completely objectively;
  - all subjects receive equal scrutiny for eligibility violations;
  - all detected violations of the particular entry criterion are excluded.

8.2.3 Reasons for exclusion from the Safety Set

Reason for exclusion of subjects from the Safety Set is the following:

- failure to take at least one dose of the IDs.

8.3 Hypothesis tests

This is an exploratory investigation and no regulatory claim will be based on the results of this investigation.

The aim of the investigation is just to collect preliminary data in order to evaluate tolerability, safety, permanence on the ocular surface and efficacy of two concentrations of Lubricin (20 and 50 µg/mL) eye drops versus sodium hyaluronate (HA) 0.18% eye drops (Vismed®) in patients with ocular discomfort following refractive surgery.

No formal hypothesis test will be performed in order to compare the two concentrations of Lubricin (20 and 50 µg/mL) eye drops versus sodium hyaluronate (HA) 0.18% eye drops (Vismed®).

8.4 Sample size and power considerations

The expected mean VAS changes, the expected standard deviations and the expected proportions of subjects reporting at least one TEAE throughout the study used for the sample size calculation are not based on any clinical result or literature data since this information is not available for the comparison of the two concentrations of Lubricin (20 and 50 µg/mL) eye drops versus sodium hyaluronate (HA) 0.18% eye drops (Vismed®) in patients with ocular discomfort following refractive surgery.

Due to the exploratory nature of the investigation, the sample size calculation is based on the confidence interval precision of the difference in the expected mean VAS changes and of the difference in the expected proportions of subjects reporting at least one TEAE throughout the study; the confidence level α is set to 0.05 for all the confidence intervals without any formal adjustment for multiplicity.

8.4.1 Confidence interval precision of the difference in the expected mean VAS changes

Supposing to have a value of -20 mm for all the mean VAS changes (dryness, foreign body sensation, burning/stinging, itching, pain, stick feeling, blurred vision and photophobia) and a standard deviation σ of 10 mm (the 50% of the expected mean VAS change) in the Lubricin 20 µg/mL arm, Lubricin 50 µg/mL arm and Vismed® 0.18% arm, the number of subjects per treatment arm required in order to have a two-sided 95% confidence interval of the difference in means with a precision ω (defined as the length of the confidence interval divided by two) of 9 mm can be calculated according to the following formula (7):

$$N_{per\ arm} = ceiling\left(\frac{2\,\sigma^2\,z^2_{1-\frac{\alpha}{2}}}{\omega^2}\right)$$

α (2-sided)	ω (precision)	σ	$N_{per\ arm}$
0.05	9 mm	10 mm	10

8.4.2 Confidence interval precision of the difference in the expected proportions of subjects reporting at least one TEAE throughout the study

Supposing to have a proportion of subjects reporting at least one TEAE throughout the study of 0.15 (i.e. 15%) in the Lubricin 20 µg/mL arm (π_{T1}) and in the Lubricin 50 µg/mL arm (π_{T2}) and of 0.45 (i.e. 45%) in the Vismed® 0.18% arm (π_C), the number of subjects per treatment arm required in order to have a two-sided 95% confidence interval of the difference in proportions with a precision ω (defined as the length of the confidence interval divided by two) of 0.4 (i.e. 40%) can be calculated according to the following formula (8):

$$N_{per\ arm} = ceiling\left(\frac{z^2{}_{1-\frac{\alpha}{2}}\left[\pi_{T_j}\left(1-\pi_{T_j}\right)+\pi_C(1-\pi_C)\right]}{\omega^2}\right)$$

α (2-sided)	ω (precision)	π_{T1}, π_{T2}	π_C	$N_{per\ arm}$
0.05	40%	15%	45%	10

8.5 Handling of missing data

Not available data will be evaluated as "missing values". All data obtained will be used in the analysis and no imputation will be carried out for missing data to come from data not recorded in the CRF, from withdrawal of subjects from the study or from exclusion of subjects evaluated during the blind review meeting.

8.6 Study/Primary eye identification

If just one eye is treated, that is the "Study/Primary eye". If both eyes are treated, the worst one (highest VAS average score) is chosen as "Study/Primary eye"; in the event average VAS score is equal in both eyes, right eye is chosen as "Study/Primary eye". The other eye is referred to as "Non-study/Secondary eye".

8.7 Demographic, baseline and background characteristics

Demographic, baseline and background characteristics will be reported for all the subjects included into the Enrolled Set.

Subjects will be analysed according to the treatment they actually received.

8.7.1 Subjects' disposition

The disposition of all subjects enrolled in the study will be listed (Listing 16.2.4.1) and summarised by treatment (Table 14.1.1.1). The number and proportion of subjects enrolled, randomized, treated and completing the study, the number and proportion of withdrawals and the reasons for withdrawal will be presented.

8.7.2 Analysis sets

The subjects included in each analysis sets will be listed (Listing 16.2.4.2) and summarised by treatment (Table 14.1.1.2).

8.7.3 Subjects excluded from efficacy and /or safety analysis

All subjects excluded from the efficacy and /or safety analysis will be listed and the reasons for exclusion will be reported (Listing 16.2.3.1).

8.7.4 Discontinued subjects

All subjects who discontinued the clinical investigation (if any) will be listed. Last device administered before discontinuation, gender, age, last visit performed before discontinuation,

time elapsed from last device administration (days), date of premature discontinuation and primary reason for subject premature discontinuation will be reported (Listing 16.2.1.1).

8.7.5 Protocol deviations

All the protocol deviations reported during the clinical investigation will be listed (Listing 16.2.2.1) and summarised by treatment. The number and proportion of subjects for each deviation will be reported (Table 14.1.1.5).

8.7.6 Blind breaking

The date and the reason of any blind breaking will be listed (Listing 16.2.2.2).

8.7.7 Demography

Demographic data will be listed (Listing 16.2.4.3) and summarised by treatment (Table 14.1.1.3). The number and proportion of subjects in each category for categorical variables (e.g. race) and descriptive statistics (mean, SD, CV%, minimum, median and maximum) for continuous variables (e.g. age, weight) will be presented.

8.7.8 Inclusion/exclusion criteria not met

All the unmet inclusion/exclusion criteria will be listed (Listing 16.2.4.4) and summarised by treatment (Table 14.1.1.4).

8.7.9 Medical, ocular and surgical history

All the diseases of medical and ocular medical history and the surgeries of all subjects enrolled in the study will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 20.1, listed (Listing 16.2.10.1) and summarised by treatment (Table 14.1.1.6). The number and proportion of subjects with any findings will be presented by PT and SOC.

8.7.10 Prior and concomitant medication

All prior and concomitant medications will be coded using the World Health Organization Drug Dictionary Enhanced (WHODDE) September 2017 and listed by treatment (Listing 16.2.10.2).

Prior medications will be summarized by treatment (Table 14.1.1.7). The number and proportion of subjects with any concomitant medication will be presented by and ATC 4th level (or the higher available ATC level if 4th level is missing) and standardised drug name.

Concomitant medications will be summarized by treatment (Table 14.1.1.8). The number and proportion of subjects with any concomitant medication will be presented by and ATC 4th level (or the higher available ATC level if 4th level is missing) and standardised drug name.

8.7.11 Subjects' study visits

The dates of all subjects study visits will be listed by treatment (Listing 16.2.10.3).

8.8 Investigational device administration at site

The date and time of device administration at site for the study/primary eye and for the non-study/secondary eye will listed by treatment and time point (Listing 16.2.5.1).

8.9 Investigational device accountability

The identifier of the dispensed kit, the dispensation date and visit, the number of dispensed multidose vials, the return date and visit and the number of returned multidose vials will listed by treatment (Listing 16.2.5.2).

8.10 Lubricin concentrations

The date and time of tears collection, the Lubricin concentrations for right eye and left eye and their changes from pre-dose values for right eye and left eye at day 1 and day 15±2 will be listed by treatment (Listing 16.2.5.3). BLQL values will be imputed as zeros for calculating the changes from pre-dose.

8.11 Efficacy analysis

8.11.1 Primary efficacy analysis

The primary efficacy analysis will be performed on the subjects included into the Full Analysis Set.

Subjects will be analysed according to the treatment they actually received.

8.11.1.1 Tolerability using a Visual Analogue Scale (VAS) for dryness, foreign body sensation, burning/stinging, itching, pain, stick feeling, blurred vision and photophobia

The VAS values of each ocular symptom (i.e. dryness, foreign body sensation, burning/stinging, itching, pain, sticky feeling, blurred vision and photophobia) and their changes from day 1 - pre-dose values and day 15±2 - pre-dose values for the study/primary eye and for the non-study/secondary eye will be listed (Listing 16.2.6.1) and summarised by treatment and time point using descriptive statistics (mean, SD, CV%, minimum, median and maximum) (Table 14.2.1.1).

The VAS values of each ocular symptom for the study/primary eye and for the non-study/secondary eye will be analysed using an analysis of variance for repeated measures model. The model will include fixed effect terms for time point, baseline covariate (i.e. the day 1 - pre-dose value) and treatment. Time point will be specified as a repeated measurement. An unstructured covariance structure of the R matrix will be used and covariance parameters will be estimated using the restricted maximum likelihood method (Table 14.2.2.1).

Only the VAS values collected at Visit 1, Visit 2 and Visit 3 will be included into the analysis of variance for repeated measure model.

8.11.2 Sensitivity analyses

No sensitivity analysis is planned.

8.11.3 Subgroups analyses

No subgroup analysis is planned.

8.11.4 Secondary efficacy analysis

The secondary efficacy analysis will be performed on the subjects included into the Full Analysis Set.

Subjects will be analysed according to the treatment they actually received.

8.11.4.1 Ocular surface vital staining with Fluorescein (Oxford scale)

The scores of ocular surface vital staining with fluorescein and their changes from day 1 - pre-dose values and day 15±2 - pre-dose values for the study/primary eye and for the non-study/secondary eye will be listed (Listing 16.2.6.2) and summarised by treatment and time point using descriptive statistics (mean, SD, CV%, minimum, median and maximum) (Table 14.2.1.2).

The changes from day 1 - pre-dose values at day 15±2 pre-dose will be compared between treatments using a Student's t test (Table 14.2.2.2).

8.11.4.2 Schirmer-I test (without anaesthesia)

The values of 8.8.4.2 Schirmer-I test (without anaesthesia) and their changes from day 1 - pre-dose values and day 15±2 - pre-dose values for the study/primary eye and for the non-study/secondary eye will be listed (Listing 16.2.6.3) and summarised by treatment and time point using descriptive statistics (mean, SD, CV%, minimum, median and maximum) (Table 14.2.1.3).

The changes from day 1 - pre-dose values at day 15±2 pre-dose will be compared between treatments using a Student's t test (Table 14.2.2.3).

8.11.4.3 Tear film break-up time

The values of tear film break-up time and their changes from day 1 - pre-dose values and day 15±2 - pre-dose values for the study/primary eye and for the non-study/secondary eye will be listed (Listing 16.2.6.4) and summarised by treatment and time point using descriptive statistics (mean, SD, CV%, minimum, median and maximum) (Table 14.2.1.4).

The changes from day 1 - pre-dose values at day 15±2 pre-dose will be compared between treatments using a Student's t test (Table 14.2.2.4).

8.11.4.4 Best corrected distance visual acuity

The values of best corrected distance visual acuity and their changes from day 1 - pre-dose values and day 15±2 - pre-dose values for the study/primary eye and for the non-study/secondary eye will be listed (Listing 16.2.6.5) and summarised by treatment and time point using descriptive statistics (mean, SD, CV%, minimum, median and maximum) (Table 14.2.1.5).

The changes from day 1 - pre-dose values at day 15±2 pre-dose will be compared between treatments using a Student's t test (Table 14.2.2.5).

8.11.4.5 Symptom assessment in dry eye

The values of symptom assessment in dry eye (frequency of symptoms and intensity of symptoms) and their changes from day 1 - pre-dose values and day 15±2 - pre-dose values will be listed (Listing 16.2.6.6) and summarised by treatment and time point using descriptive statistics (mean, SD, CV%, minimum, median and maximum) (Table 14.2.1.6).

The changes from day 1 - pre-dose values at day 15±2 pre-dose will be compared between treatments using a Student's t test (Table 14.2.2.6).

8.11.4.6 Slit lamp examination

The scores of slit lamp examination (Eyelid - Meibomian glands, Eyelid - Erythema, Eyelid - Oedema, Lashes, Conjunctiva - Erythema, Conjunctiva - Oedema, Lens, Iris, Anterior Chamber Inflammation, Cornea transparency and Cornea neovascularization) and their changes from day 1 - pre-dose values and day 15±2 - pre-dose values for the study/primary eye and for the non-study/secondary eye will be listed (Listing 16.2.6.7) and summarised by treatment and time point using descriptive statistics (mean, SD, CV%, minimum, median and maximum) (Table 14.2.1.7).

The changes from day 1 - pre-dose values at day 15±2 pre-dose will be compared between treatments using a Wilcoxon Rank Sum Test (Table 14.2.2.7).

8.11.4.7 Intraocular pressure

The values of intraocular pressure and their changes from day 1 - pre-dose values and day 15±2 - pre-dose values for the study/primary eye and for the non-study/secondary eye will be listed (Listing 16.2.6.8) and summarised by treatment and time point using descriptive statistics (mean, SD, CV%, minimum, median and maximum) (Table 14.2.1.8).

The changes from day 1 - pre-dose values at day 15±2 pre-dose will be compared between treatments using a Student's t test (Table 14.2.2.8).

8.11.4.8 Corneal sensitivity by Cochet-Bonnet aesthesiometry

The values of corneal sensitivity by Cochet-Bonnet aesthesiometry and their changes from day 1 - pre-dose values and day 15±2 - pre-dose values for the study/primary eye and for the non-study/secondary eye will be listed (Listing 16.2.6.9) and summarised by treatment and time point using descriptive statistics (mean, SD, CV%, minimum, median and maximum) (Table 14.2.1.9).

The changes from day 1 - pre-dose values at day 15±2 pre-dose will be compared between treatments using a Student's t test (Table 14.2.2.9).

8.11.5 Interim analyses

No interim analysis is planned.

8.12 Safety and tolerability analysis

The safety and tolerability analysis will be performed on the subjects included into the Safety Set.

Subjects will be analysed according to the treatment they actually received.

8.12.1 Adverse events

Adverse events (AEs) will be coded by System Organ Class (SOC) and Preferred Term (PT), using the Medical Dictionary for Regulatory Activities (MedDRA) version 20.1.
AEs will be classified as pre-treatment AEs (PTAEs) and treatment-emergent AEs (TEAEs), according to the period of occurrence, as follows:

- PTAEs: all AEs occurring before the first dose of the ID and not worsening after the first dose of the ID
- TEAEs: TEAEs: all AEs occurring or worsening after the first dose of the ID

Individual PTAEs and TEAEs will be listed by treatment (Listing 16.2.7.1, Listing 16.2.7.2).

No summary table will be provided for PTAEs.

TEAEs will be summarised by treatment and overall.

- The number and percentage of subjects with any TEAE and the number of TEAEs will be presented overall (Table 14.3.1.1) and by SOC and PT (Table 14.3.1.2);
- The number and percentage of subjects with any TEAE by relationship and the number of TEAEs by relationship will be presented (Table 14.3.1.1);
- The number and percentage of subjects with any TEAE by intensity and the number of TEAEs by intensity will be presented overall (Table 14.3.1.1) and by SOC and PT (Table 14.3.1.3);
- The number and percentage of subjects with any TEAE related to the ID (Table 14.3.1.1) and the number of TEAEs related to the ID will be presented overall and by SOC and PT (Table 14.3.1.4);
- The number and percentage of subjects with any TEAE leading to discontinuation and the number of TEAEs leading to discontinuation will be presented (Table 14.3.1.1).

Serious TEAEs will be summarised by treatment and overall.

- The number and percentage of subjects with any Serious TEAE and the number of Serious TEAEs will be presented overall (Table 14.3.1.1) and by SOC and PT (Table 14.3.1.5);
- The number and percentage of subjects with any Serious TEAE by relationship and the number of Serious TEAEs by relationship will be presented (Table 14.3.1.1);
- The number and percentage of subjects with any Serious TEAE related to the ID and the number of Serious TEAEs related to the ID will be presented overall and by SOC and PT (Table 14.3.1.6);
- The number and percentage of subjects with any Serious TEAE leading to discontinuation and the number of Serious TEAEs leading to discontinuation will be presented (Table 14.3.1.1).

All TEAEs leading to death will be listed, all Serious TEAEs will be listed and all TEAEs leading to discontinuation will be listed (Table 14.3.2.1).

8.12.2 Device deficiencies

The record dates and the descriptions of the device deficiencies will be listed by treatment (Listing 16.2.7.3).

8.12.3 External ocular examination

The date of external ocular examination, the overall investigator's interpretation (Normal; Abnormal, Not Clinically Significant; Abnormal, Clinically Significant) and clinically significant abnormalities (if any) for the study/primary eye and for the non-study/secondary eye will be listed by treatment and time point (Listing 16.2.9.1).

8.12.4 Review of ocular medical condition

The date of review ocular medical condition, the overall investigator's interpretation (Normal; Abnormal, Not Clinically Significant; Abnormal, Clinically Significant) and clinically significant abnormalities (if any) will be listed by treatment and time point (Listing 16.2.9.2).

8.12.5 Review of systemic medical condition

The date of review systemic medical condition, the overall investigator's interpretation (Normal; Abnormal, Not Clinically Significant; Abnormal, Clinically Significant) and clinically significant abnormalities (if any) will be listed by treatment and time point (Listing 16.2.9.3).

8.13 Analysis datasets

Analysis datasets will be created according to the version 2.1 of the ADaM model of CDISC (9).

9 REFERENCES

1 ICH Topic E6 (R1) - Guideline for Good clinical practice

2 ICH Topic E6 (R2) - Integrated Addendum to ICH E6 (R1) - Guideline for Good clinical practice

3 ICH Topic E9 - Statistical Principles for Clinical Trials

4 ISO14155-2012 - Clinical investigation of medical devices for human subjects - Good clinical practice

5 Clinical Investigational Plan LUB0116MD. "A 2 week, randomized, double-masked, controlled, parallel group study to evaluate tolerability, safety, permanence on the ocular surface and efficacy of two concentrations of Lubricin (20 and 50 µg/mL) eye drops versus sodium hyaluronate (HA) 0.18% eye drops (Vismed®) in patients with ocular discomfort following refractive surgery". Final version 2.0, 13DEC2016

6 SAS/STAT® User's Guide, Version 9.3 (TS1M1) for Windows

7 Dixon, W.J., Massey, F.J. (1983) Introduction to Statistical Analysis. 4th Edition. McGraw-Hill. Pages 80-85 and 130-131

8 Dixon, W.J., Massey, F.J. (1983) Introduction to Statistical Analysis. 4th Edition. McGraw-Hill. Pages 286-288

9 CDISC Analysis Data Model Version 2.1

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