KEDRION
BIOPHARMA

STATISTICAL ANALYSIS PLAN

Protocol KB046 Amendment 7 October 2019

A Historically-Controlled Phase II/III study to Evaluate

Efficacy and Safety of Kedrion Human Plasminogen Eye Drop Preparation in Patients

Diagnosed with Ligneous Conjunctivitis

Date: 09 November 2020

Version: 4.0

REVIEW AND APPROVAL

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Kedrion S.p.A

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List of Abbreviations

Abbreviation*	Term
ADR	Adverse Drug Reaction
Ab	Antibody
AE	Adverse Event
Ag	Antigen
ALT	Alanine Aminotransferase/Transaminase
BUN	Blood urea nitrogen
CA	Competent Authority
CBC	Complete Blood Count
eCRF	Electronic Case Report Form
EC	Ethics Committee
EDTA	Ethylenediaminetetraacetic Acid
EMA	European Medicinal Agency
EU	European Union
FDA	Food and Drug Administration
FFP	Fresh Frozen Plasma
GCP	Good Clinical Practice
Hct	Hematocrit
Hgb	Hemoglobin
HAV	Hepatitis A Virus
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIPAA	Health Insurance Portability and Accountability Act
HIV-1	Human Immunodeficiency Type 1 Virus
HIV-2	Human Immunodeficiency Type 2 Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IU	International Unit
IUD	Intrauterine Device
IMP	Investigational Medicinal Product
IND	Investigational New Drug
Kg	Kilogram
LC	Ligneous Conjunctivitis
MedDRA	Medical Dictionary for Regulatory Activities

Abbreviation*	Term
ml	Milliliter
NAT	Nucleic acid test
NIH	National Institute of Health
Nr	Number
NTF	Note to File
OD	Optical Density
PI	Principal Investigator
PT	Preferred Terms
PVD	Pharmacovigilance Department
QA	Quality Assurance
RBC	Blood Cell Count
SADR	Serious Adverse Drug Reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOC	System Organ Class
SUSAR	Suspected Unexpected Serious Adverse Event Reporting
TSE	Transmissible Spongiform Encephalopathy
US	United States
WBC	White Blood Cells

* This is a list of abbreviations frequently used in Kedrion S.p.A. protocols. All of these abbreviations may or may not be used in SAP.

1. Introduction

This Statistical Analysis Plan (SAP) describes the planned analysis and reporting for protocol KB046, entitled, “A Historically-Controlled Phase II/III study to Evaluate Efficacy and Safety of Kedrion Human Plasminogen Eye Drop Preparation in Patients Diagnosed with Ligneous Conjunctivitis”, Amendment 8, dated April 02, 2020. The purpose of this SAP is to describe the statistical methodologies that will be used to address the objectives of the above study and come to conclusions regarding the study objectives, ensuring their validity and suitability.

2. Study Objectives and Endpoints

2.1 Primary Objective

1. Evaluation of the efficacy of the IMP, Kedrion Human Plasminogen eye drop preparation, for the treatment of LC associated with Type I plasminogen deficiency in symptomatic subjects measured by recurrence of pseudomembrane after complete regression due to surgery or treatment with the IMP (Segment 2).
2. Evaluation of the safety of the IMP in symptomatic subjects and asymptomatic subjects with a history of ocular pseudomembranes.

2.2 Secondary Objective

1. Evaluation of the efficacy of the IMP in the regression of pseudomembranes in symptomatic subjects (Segment 1).
2. Assessment of the local tolerability of the IMP.
3. Assessment of the immunogenicity of the IMP.

2.3 Primary Efficacy Endpoint

The prevention of pseudomembrane recurrence during Segment 2 after:

- Surgical removal of pseudomembranes, in cases where there is no pseudomembrane regression (<20%) or partial regression, (between 20% and 90%) following initial treatment with the IMP (Group 1B); or
- Complete pseudomembrane regression (>90%), during Segment 1 in response to treatment with the IMP (Group 1A).

The primary efficacy endpoint will be assessed during Segment 2.

The pseudomembrane recurrence will be assessed by 2 independent evaluators based on pictures and data collected during the visits.

The primary efficacy endpoint will be classified using a categorical scale with 3 levels: complete success, defined as no recurrence by the end of Segment 2; partial success, defined as recurrence appearing 2 weeks or more after the start of Segment 2, or if following the 3rd cycle of Segment 2 for Group 1A no recurrence occurs maintaining the higher dose; or failure, defined as recurrence within 2 weeks of the start of Segment 2 or if at repeat cycles of Segment 1 for Group 1A, the pseudomembrances do not regress after Segment 1.

The clinical course of each eye during the study, including pseudomembranes regression and recurrence, will be compared with the eye's clinical history prior to study entrance as its own historical control specific for left or right eye, dependent on the data available. If not available the standard expectation of the independent assessors will be used. Even if only one of the two eyes affected with pseudomembrances recurs, it will be treated as a treatment failure. Treatment with the IMP was considered effective if the rate of pseudomembrane recurrence was lower than the recurrences in the subjects' historical data, with each evaluated eye compared with its own history of recurrence.

2.4 Secondary Efficacy Endpoints

- Regression in pseudomembrane surface areas (PSA) of existing ligneous pseudomembranes (using objective measurement) at the end of Study Segment 1, defined as:
  - Complete Regression: PSA reduction greater than 90%;
  - Partial Regression: PSA reduction between 20% and 90%;
  - No Regression: PSA reduction of less than 20%.

- Time to ligneous pseudomembrane re-appearance after surgery or complete regression (>90%) (days) during Segment 2.

Secondary efficacy endpoints will also be compared with each eye's clinical history.

The regression and/or recurrence of soft/new membranes will be measured by 2 independent evaluators based on a calculation of surface area [redacted] which allows the user to hand-draw an area around the lesion from an imported photograph and, when a scale bar is used, it will calibrate and immediately calculate the size of the drawn area.

In case when the measurements with [redacted] are missing or impossible to be translated into surface area measured in mm^2 (e.g. photographs taken at different distances without a scale bar - ruler -; a development in thickness of the membranes or other reasons the analysis will be committed to a non-

treating independent reviewer. In this case, the non-treating independent reviewer will repeat also the Primary Efficacy Assessment, allowing a standardization of the overall efficacy evaluation criteria in the trial (see "Method for Independent Review and Assessment of Primary and Secondary Efficacy Endpoints" in attachment).

A lack of regression of membranes in Segment 1 will not constitute treatment failure, but will offer eligibility for surgery and transition to Study Segment 2, in Group 1B.

2.5 Safety Endpoints

Clinical safety will be assessed in all subjects (Segment **1&2,**and continuation segment) receiving any dose of the IMP by evaluating:

General Safety

- Percentage of subjects who experienced any AEs, including laboratory. From receiving the first dose and throughout the study, all AEs will be collected whether related or not and coded. AEs whose causality have been assessed as possible, probable or definitely related to the IMP, will be considered IMP-related events and upgraded to Adverse Drug Reactions (ADR).
- Changes in Vital signs, including blood pressure, heart rate, temperature, respiratory rate.
- Changes in standard safety laboratory (hematology and clinical chemistry) testing.

Local tolerability.

- Percentage of subjects who experienced signs and symptoms of sensitization;

Immunogenicity

- Percentage of subjects who developed antibodies against human plasminogen;
- Percentage of subjects who developed antibodies against bovine aprotinin;

Immunogenicity studies will be carried out as part of the safety assessment. Blood samples will be taken before starting the treatment at study entry, at the end of Segment 1, and every 4 weeks thereafter in Segment 2 as well as every 2 months during Continuation Segment (Starting from Protocol Amendment 4 implementation) and Termination Visits 1 and 2.

Viral Safety

A pre-treatment serum sample from each subject included in the clinical trial will be stored at a temperature below -70°C for possible future viral testing. A post-treatment serum sample, from each subject who received the IMP at least once, will be collected at the termination visit for comparison.

3. Study Design and Procedures

For detail study design, please refer to the study protocol section 6.1: “Overall Study Design and Plan Description.” This will be an open label, historically-controlled, multicenter Phase II/III study. The study will be divided in three segments:

Study Segment 1: Open label, historically-controlled evaluation of the efficacy and safety of the IMP in symptomatic (Group 1) and asymptomatic (Group 2) subjects with LC.

Study Segment 2: Open label, historically-controlled evaluation of the efficacy and safety of the IMP in preventing the recurrence of pseudomembranes following surgery or complete regression after treatment with the IMP.

Continuation Segment: Open label evaluation of long-term safety of the IMP subjects with one or more eyes demonstrating efficacy success (from Groups 1A and 1B) or those who remain asymptomatic (Group 2).Two additional subjects could be enrolled end included in continuation segment only. Subjects who have withdrawn from the ongoing study will not be replaced.

In this study the subjects will serve as their own controls, and treatment effects will be compared with the subject’s own disease and treatment history, as available. Because LC is a very rare condition with an estimated prevalence of 1.6 per 1,000,000 people (Schuster et al., 1999), the scarcity of LC patients and a lack of approved treatment preclude the use of a randomized, controlled design.

Table 1 details the regularly scheduled visits and measurement time at each visit.

able 1: Schedule of Study Procedures

Day	Up to Week 4	Week 0	Week S1-4	Surgery window SW1–2	2 weeks post surgery	Week S2-4	Week S5-8	Unsche-duled Visits	Post-Exit Follow-Up Week 24	Every 2 months during Continuati on Segment	Every 6 months after the start of the Continuation Segment	End of the Study
Visit	Screening Visit	Baseline Visit	Visit 1	SV	PSV	Visit 2	Visit 3/T1	UV	Visit 4		Visit	Visit /T2
Informed Consent/Assent	X											
Inclusion/Exclusion Criteria	X											
Medical History	X											
Prior and Concomitant Therapy							X					
Subject Diary*		X*	X*	X*	X*	X*	X*		X*		X	X*
Physical Assessment and Vital Signs (blood pressure, pulse, respiratory rate and body temperature)	X	X	X	X	X	X	X	X	X		X	X
Height and Weight	X	X	X	X	X	X	X	X	X		X	X
Urine Pregnancy Test (females only)	X										X	
Ophthalmology examination	X	X	X	X**	X	X	X	X	X		X	X
Photograph and measurement of pseudomembranes, if present	X	X	X	X	X	X	X	X	X		X	X
Blood sample for hematology and clinical chemistry	X		X			X	X				X	X

Day	Up to Week 4	Week 0	Week S1-4	Surgery window SW1–2	Within 2 weeks of surgery	Week S2-4	Week S5-8	Unsche-duled visit	Post-Exit Follow-Up Week 24	Every 2 months during Continuati on Segment	Every 6 months after the start of the Continuation Segment	End of the Study
Enrollment/Assignment of subject ID	X											
Blood sample for pre-treatment sample storage		X										
Blood sample for Plasminogen activity and Plasminogen antigen test	X											
Immunization for Hepatitis A and/or B	X											
Dispense IMP kit and subject diary		X	X	X	X	X	X	X			X	
Blood sample for viral safety testing	X						X					X
Blood sample for immunogenicity testing	X		X	X		X	X			X	X	X
Collect IMP kit and subject diary			X			X	X		X		X	X
Blood sample for genetic determination		X										
Perform IMP Accountability			X	X		X	X	X	X***			X
Adverse Events							X					

S1: Segment 1. SW: Surgery window. SV Surgery Visit. PSV Post Surgery Visit. UV Unscheduled Visit. S2: Segment 2. C: Continuation Segment.

* Site will call the subjects weekly to follow up on subject status and remind the subject about completing the Subject diaries to be collected each month at site visits. After Segment 2 is complete and subject enters the continuation phase the monthly calls will continue, but the diaries will be mailed in.

** Two photographs will be taken before and after surgery (if possible)

*** used and unused vials will be mailed into the site monthly

Additional Subjects enrolled in the Continuation Segment will undergo the Screening Baseline, follow up visits every 6 months and Visit Term#2 assessments

4. Study Medication

The Investigational Medical Product (the IMP) for this study will be Kedrion Human Plasminogen which is a sterile human plasma-derived plasminogen preparation in the pharmaceutical form of an eye drop solution for topical ocular use, with a total protein concentration of 1 mg/ml, of which at least 93% is plasminogen. As specified in the protocol, study medication will be administered only to subjects who have signed and dated an informed consent.

The route of administration is topical ocular application, utilizing a drop volume of 0.05 mL.

The given dose varies based on the symptoms presented (e.g. pseudomembranes present) and the course of treatment (e.g. surgery). The dosage is detailed in Table 2.

Table 2: Dose Regimen

Segment	Subject group	Dose (per eye)	Frequency (per day)
Study Segment 1	Group 1 (pseudomembranes)	2 drops	8 times
	Group 2 (no pseudomembrane)	2 drops	6 times
Surgery Window	Group 1B	2 drops	8 times
Study Segment 2	Group 1A (no surgery) 1st and 2nd cycles Group 1A (no surgery) 3rd cycle	2 drops 2 drops	6 times 8 times
	Group 1B (post-surgery)	2 drops	Descending frequency: 12 times (Week S2-1) 8 times (Week S2-2 to S2-4) 6 times (Week S2-5 to S2-8)
	Group 2 (no pseudomembrane)	2 drops	6 times
Continuation Segment	Group 1A (cycles 1 and 2) and 1B subjects with efficacy success Group 2 (no pseudomembrane) Additional asymptomatic subjects	2 drops	4 to 6 times*
Continuation Segment	Group 1A (3rd cycle)	2 drops	8 drops

*dose at the discretion of the Investigator.

The above dose regimen was based on published literature and clinical experience (Watts et al. 2002; Heidemann et al. 2003; Caputo et al. 2008). These studies describe a prompt and dramatic response resulting in complete resolution of the soft membranes and absence of recurrences after surgical removal of the hard membranes after administration of 2 drops/eye plasminogen initially hourly or every two hours, with subsequent reduction in the number of doses administered per day:

Treatments should be applied daily at evenly spaced intervals during waking hours.

Treatment dose (Group **1**, subjects with pseudomembranes, Group 1A during Study Segment 2, 3rd cycle)

- 2 drops per eye for 8 administrations per day during waking hours, approximately every 1.5 hours.

Maintenance dose (Group 2, subjects with no pseudomembranes and Group **1A** during Study Segment 2, 1st and 2nd cycles)

- 2 drops per eye for 6 administrations per day during waking hours, approximately every 2 hours.

Post-surgery descending dose regimen (Group **1B** in Study Segment 2)

- 2 drops per eye for 12 administrations per day during waking hours, approximately every 1 hour.
- 2 drops per eye for 8 administrations per day during waking hours, approximately every 1.5 hours.
- 2 drops per eye for 6 administrations per day during waking hours, approximately every 2 hours.

Continuation Segment dose

Dose is selected by the Investigator 2 drops per eye between 4-6 times a day (Group 1A cycles 1 and 2, 1B and Group 2 including the additional subjects enrolled in the continuation segment), or 2 drops per eye 8 times/day, Group 1A cycle 3.

- 2 drops per eye for 8 administrations per day during waking hours, approximately every 1.5 hours, is recommended
- 2 drops per eye for 6 administrations per day during waking hours, approximately every 2 hours, is recommended.

- 2 drops per eye for 5 administrations per day during waking hours, approximately every 2.5 hours is recommended.
- 2 drops per eye for 4 administrations per day during waking hours, approximately every 3 hours.

5. Statistical Method and Determination of Sample Size

5.1 Analysis Populations

Intent-to-Treat (ITT):

ITT population will consist of all eyes of subjects who enrolled in the study (Segment 1 and Segment 2) and who receive at least one dose of the study treatment.

Modified ITT (mITT):

mITT population will consist of all eyes assigned to Groups 1A and 1B at the start of Study Segment 2, who received at least one dose of the study treatment, and had at least one efficacy assessment in Segment 2.

Per-Protocol Population

Per-Protocol population will include all eyes included in mITT who have completed both Segment 1 and segment 2 of the study, and have completed at least 80% of the protocol-required doses of the IMP without any major protocol violations or exception that could impact the integrity of study data.

Efficacy analyses will be based on mITT and per-protocol population.

Safety Population:

The safety population will consist of all subjects enrolled who receive at least one dose of the study treatment.

5.2 Data Analysis

All the baseline, safety and efficacy endpoints will be summarized using descriptive statistics (mean, standard deviation, median, 25th percentile and 75th percentile [for quantitative parameters] and proportion [for qualitative variables]). Historical data on subjects' past LC treatment before enrolling in this

study, including surgical and medical treatment received, response to the treatment, pseudomembrane regression, recurrence, and time to recurrence, will be presented descriptively (without statistical comparisons).

5.3 Determination of Sample Size

Due to very low disease prevalence, no formal sample size calculations were completed. Instead a target sample size of at least 10 subjects, for approximately of 20 evaluable eyes, was selected by the treating investigators as a reasonable size to evaluate the efficacy endpoints, as well as the safety of the IMP in patients with Ligneous Conjunctivitis.

5.4 Efficacy

Efficacy responses will be summarized and presented on the basis of individual eyes. The primary endpoint (recurrence rate) will be presented descriptively as a frequency table based on the categorization described in Section 6.1.1 of the protocol. Confidence intervals for the recurrence rate (complete success, and complete plus partial success) will be calculated on the assumption . The time to recurrence will also be presented using a Kaplan-Meier survival plot. Shift tables will be presented by subjects' history of LC treatment and pseudomembrane regression and recurrence with study data. No statistical comparisons will be conducted.

The secondary endpoint (regression rate) will also be presented as a frequency table, showing the numbers of eyes with complete, partial, or no regression during Study Segment 1.

In addition, the relapse rate (number of subjects who present ligneous membranes whose overall surface area increased or whose pseudomembranes reappeared after regression) will be reported.

Efficacy responses will be tabulated for both the mITT and per-protocol populations (if different).

5.5 Safety

The assessment of safety will be based on individual study subjects, except for local tolerability assessments that will be based on individual eyes.

The safety will be determined with a focus on the percentage of subjects who experience treatment-related (i.e., those rated as “possibly related,” “probably related” or “definitely related”) AEs (i.e., ADRs). Each local and systemic reaction will be registered and the percentages of subjects reporting each reaction will be reported.

A complete listing of all safety data will be provided. Adverse experiences, vital signs, reasons for study termination and laboratory data will be summarized. Rates of occurrence of adverse experiences will be calculated and concomitant medications will be listed. The probability of adverse events over time will be described using the Kaplan-Meier survival method. The time of occurrence will be calculated in each subject as the difference from the date of adverse event and the date of enrollment into the study. Those subjects, not experiencing the event, will be censored at the last observation date.

AEs will be coded using MedDRA® version 17.0 or later, and will be updated to the latest version prior to database lock. The incidences of AEs rated as “possibly related,” “probably related” or “definitely related” will be summarized for each product group by Preferred Terms System (PT) and System Organ Class (SOC). If a subject experienced multiple AEs that mapped to the same preferred term, the AE will be counted only once using the highest severity and closest IMP relationship. Incidences of all AEs and AEs that are unrelated or unlikely to be related to the IMP will also be summarized separately. SAEs will be listed by subject. Data listings of all AEs will be provided.

Local tolerability will be provided in data listing.

Viral Safety

A pre-treatment serum sample will be obtained at the screening visit and stored at -70ºC or below for possible future testing. A final blood sample will be collected at Termination visit 1 (Visit 3/T1) and at Termination Visit 2 (T2) and tested by serology (HIV1-2, HCV, PV-B19, HBV and HAV) and by NAT (HBV, HCV and HIV1-2; PVB19 and HAV will be tested only in case of negative serology).

Number of subjects with conversion from negative will be summarized. In addition, viral safety data will be provided in data listing.

5.6 Immunogenicity Analyses

Immunogenicity studies will be carried out as part of the safety assessment. Samples will be taken before starting the treatment at study entry, at the 4 weeks evaluation, and every 4 weeks thereafter in Segment 2, as well as every 2 months during continuation (starting from Protocol Amendment 4 implementation) and Termination Visits 1 and 2. The following markers will be measured: 1) antibodies against human plasminogen and 2) antibodies against bovine aprotinin. Timing of samples withdrawal and evaluated parameters are specified in the Schedule of procedures.

The calculation of immunogenicity statistics will be based on the number of subjects in which a detectable level of antibodies against human plasminogen and bovine aprotinin is found. The probability of antibody development over time will be described using the Kaplan-Meier survival method. The time to development will be calculated in each subject as the difference in antibody levels from the date of antibody development to the date of enrollment into the study. Those subjects not showing a change in antibody levels will be censored at the last observation date.

5.7 LC Related Historical Data Analyses

The following additional LC related historical data will be analyzed using mITT and PP population. All LC related historical data will be provided in a per-patient profile listing.

- Historical data for given subject will be listed in subject data listing, and will be included in patient profiles. Time of the first diagnosis to the time of screening for KB046. LC duration (months) at time of screening since the first diagnosis of LC will be summarized as well..
- The available historical data for all past treatments will be included in patient profiles. The following time to LC recurrence analyses will be reported separately from the medical treatment, including the outcome to these treatments. Different medical agents will be analyzed separately for each agent/class of agent, as well as combined.
    - Time to LC recurrence following a surgical psudomembrane removal will be summarized using Kaplan-Meier method. Time to LC is defined as days from date of surgery to the date of post operational recurrence. Number of eyes experienced recurrence, number of eyes censored where applicable, 25, 50, 75 percentiles (including 95% Cis) and range will be reported. Eyes that did not have recurrence will be censored at the date of screening.

- Of those who received medical treatment, time to LC recurrence following a medical treatment will be summarized using Kaplan-Meier method, by each treatment agents and all treatment agents combined. Time to LC is defined as days from the last dose of treatment to the date of post treatment recurrence. Number of eyes experienced recurrence, number of eyes censored where applicable, 25, 50, 75 percentiles (including 95% Cis) and range will be reported. Eyes that did not have recurrence will be censored at the date of screening.

5.8 Segments Repetition

In the case of a subject reports a recurrence during Segment 2 (Group 1A, 1B and 2), the repetition of Segment 1 and 2 will be offered. In particular:

GROUP 1A

If at any time during Segment 2 the subject reports recurrence of a pseudomembrane subjects will be re-entered into Group 1 Segment 1 again. A subject can recycle through Group 1 and 1A two more times. If at repeat cycles of Segment 1, the pseudomembranes do not regress after Segment 1, the subject will be deemed a failure and exit the study. If at the end of the second cycle of Segment 1 regression has occurred but recurrence occurs in second repeat of Segment 2 (lower dose), on the third cycle of Segment 2 the subject will remain on the higher dose (2 drops/ eye 8 times/day). If no recurrence occurs once maintained on the higher dose it will be considered a partial success. If a recurrence occurs at the higher dose in Segment 2, the subject will be considered a failure and the subject must exit the study.

GROUP 1B

For eyes developing pseudomembrane recurrence after surgery, the time to recurrence will be recorded. Eyes with recurrence within 2 weeks of surgery will be considered treatment failures and exit the study. Subjects with an eye(s) developing pseudomembrane recurrence more than 2 weeks after surgery will be considered as partial success and given the option to repeat the IMP treatment regimen of Study Segment 2 (descending dose).

GROUP 2

If a Group 2 eye develops pseudomembrane recurrence at any time during Segment 2, the subject will be transferred to Group 1 and - if the subject meets all inclusion/exclusion criteria - re-start Study Segment 1.

In this case the eyes will be summarized under Group 2 when receiving Group 2 regimen, and under Group 1 while receiving Group 1 regimen. After the subject complete segment 1 group 1 and re-enter segment 2, the subject will be summarized under Group 1A or 1B for segment 2.

As reported in section 2.3 the primary efficacy endpoint will be classified using a categorical scale with levels: complete success, defined as no recurrence by the end of Segment 2; partial success, defined as recurrence appearing 2 weeks or more after the start of Segment 2, or if following the 3rd cycle of Segment 2 for Group 1A no recurrence occurs maintaining the higher dose; or failure, defined as recurrence within 2 weeks of the start of Segment 2 or if at repeat cycles of Segment 1 for Group 1A, the pseudomembrances do not regress after Segment 1.

5.9 Continuation Segment Data

5.9.1 Definitions:

End of Study: is defined as the last safety follow-up information (immunogenicity results) received by investigators.

Lack of efficacy: defined as evidence of psudomembrane recurrence at any time during the Continuation Segment in a patient who had been adequately compliant with the protocol-specified study treatment regimen. Adequate compliance is defined as at least 80% of the planned doses administered.

The Continuation Segment data will be analyzed using all subjects who received any study treatment in the Continuation Segment.

All subjects included in Continuation Segment will be reported under one column, termed “All.” There will not be separate columns of subjects who participate in Segment 1 or 2.

Visit Window: To include unscheduled visit data in the reporting, visit windows will be used to calculate 6 Months follow up visit.

- For subjects who have participated in Segment 2 and continue to the Continuation Segment, his/her Visit 3/T1 study date will be used as the reference date for the visit windowing calculation.
- For subjects who directly participate in the Continuation Segment, his/her Week 0 baseline visit date will be used as the reference data for the visit windowing calculation.

All visit data after the reference dates will be used and reported. Calculated 6 month visits will be defined as follows:

Calculated Visit	Starting date (exclusive)	Ending date (inclusive)	Target visit date
1st 6-month visit	Reference date (Visit 3/T1 or Week 0 baseline)	End of 9 months	6 months from reference date
2nd 6-month visit	End of 9 months	End of 15 months	12 months from reference date
3rd 6-month visit	End of 15 months	End of 21 months	18 months from reference date
4th 6-month visit	End of 21 months	End of 27 months	24 months from reference date
5th 6-month visit	End of 27 months	End of 33 months	30 months from reference date
Based on the above algorithm, continue to calculate additional visits as needed.			

It is assumed there are 30.4375 days a month. The above table will be implemented based on the visit dates as follow:

- "1st 6-month Visit": Reference Date < Visit Date <= Reference Date + 30.4375*9
- "2nd 6-month Visit": Reference Date + 30.4375*9 < Visit Date <= Reference Date + 30.4375*15
- "3rd 6-month Visit": Reference Date + 30.4375*15 < Visit Date <= Reference Date + 30.4375*21
- "4th 6-month Visit": Reference Date + 30.4375*21 < Visit Date <= Reference Date + 30.4375*27
- "5th 6-month Visit": Reference Date + 30.4375*27 < Visit Date <= Reference Date + 30.4375*33
- Based on the above algorithm, continue to calculate additional visits as needed.

5.9.2 Subject Disposition

Subject disposition information will be summarized for all subjects. Summaries will include: the number of subjects continued from the main study and subjects who entered in the study staring from Continuation Segment.

The date of termination/discontinuation including primary reason for discontinuation will be also included.

5.9.3 Demographic

Subjects' demographic including age, sex and race will be summarized.

5.9.4 Treatment Exposure

Number of months on treatment, total number of treatment vials dispensed, total number of treatment vials returned will be summarized.

5.9.5 Adverse Events

Adverse events data will be coded with MedDRA® version 17.0 or later and summarized similarly as summarized in Segment 1 and Segment 2. Analysis will include the following:

- Overview of adverse events
- AE incidence by SOC and preferred terms
- AE incidence by severity and relationship to the study treatment
- Serious AEs
- Local Tolerability AEs

5.9.6 Other Analyses

All data collected during the Continuation Segment will be provided as subject listings, which will include the following as applicable:

- Subject disposition
- Protocol deviations
- Concomitant medications
- Medical and LC history
- Laboratory tests
- Plasminogen activity and antigen tests
- Drug dispensation and accountability
- Adverse events
- Pregnancy data, if available
- Patient diary data

6. Data Preparation

For each subject, an eCRF will be supplied. The amendments to eCRF data may be performed according to the instruction in the OpenClinica Database which will keep and audit trail. The original text should remain legible.

All data collected will be entered into a database and later analyzed. The study data should be verified with the original data, thereafter all the records, laboratory tests and clinical records of the subjects will be

accessible. The investigator should allow access to the subject clinical records and the original study data should always be available for review.

All analyses outlined in this document will be carried out after:

- All data management requirements are met according to the SOPs, including data entry, performance of edit and validation checks, documentation and resolution of data queries, achievement of acceptably low data error rates and database lock with written authorization provided by appropriate personnel;
- Protocol deviations have been identified and related actions completed.

7. Analysis Variables

7.1 Efficacy Variables

- PMR – pseudomembrane recurrence during Segment 2 (subject % will be calculated) (Primary)
- PSA – pseudomembrane surface areas (% decrease from baseline to the end of Segment 1) (Secondary)
- ROMA – reduction of the overall membranes area at the end of Segment 1 (eye % will be calculated) (Secondary)
- TTLPR – time to ligneous pseudomembrane re-appearance after surgery or complete regression (measured in days) during Study Segment 2 (Secondary)

The primary and secondary efficacy endpoints will be assessed independently following the method and procedures described in the 'Method for Independent Review and Assessment of Primary and Secondary Efficacy Endpoints' document.

7.2 Safety Variables

Safety analysis population will include all subjects who receive at least one dose of IMP. The percentage of subjects with safety endpoints below will be calculated.

- Vital signs, including blood pressure, heart rate, temperature, respiratory rate
- AAHP – antibodies against human plasminogen
- AABA – antibodies against bovine aprotinin
- SSOS – signs and symptoms of sensitization

- Adverse Events (correlation with the IMP assessed as possible, probable, or definite)
- Change from negative to positive in viral safety testing

8. General Considerations

This study will be reported based on the standard of Clinical Data Interchange Standards Consortium (CDISC). Study raw data will be mapped using the structure of Study Data Tabulation Model (SDTM). Study Data Reviewer's Guide and Study Data Definitions will be provided. Analysis datasets will be derived using the structure of Analysis Data Model (ADaM). Analysis Data Reviewer's Guide and Analysis Data Definitions will be provided.

8.1 Derived Variables

The following derived and computed variables have been initially identified as important for the analysis of the primary and secondary target variables. It is expected that additional variables will be required. The SAP will not be amended for additional variables that are not related to the primary target or key secondary target variables. Any additional derived or computed variables will be identified and documented in the SAS programs that create the analysis files.

Variable Name	Description	Valid Values (Ranges)	Computation Methods, Notes, or Equation(s)
Age (years)	Age at Dosing	N/A	Integer part of (first dose date - birth date +1)/365.25.
RPSA	% reduction of the overall membranes area at the end of Segment 1	> 0 and < 1	RPSA=(PSABase – PSASegment1)/PSABase
TTLPR	Time to ligneous pseudomembrane re-appearance	> 0	TTLPR=LP Recurrence Date – Surgery Date or Complete Regression Date

8.2 Missing Data

For the efficacy analysis, the Last Observation Carried Forward (LOCF) method will be used to impute missing values. Pre-treatment values will be not imputed post-treatment, i.e. if observations immediately following treatment are missing, values prior to treatment will not be carried forward and the assessment

will be treated as missing. If baseline value is missing, screening value will be imputed to baseline. In order to assess the impact of missing data, sensitivity analyses without imputation (completed subject analyses) will be also performed.

8.3 Study Centers

The study is planned to be conducted using multicenters. Data will be pooled from all study centers (US and EU).

8.4 Data Review

Clinical personnel at each study center are responsible for assuring that the protocol is followed and eCRFs are properly completed. Data management procedures, including database design, development of the data dictionary, and coding of all adverse events and medications, edit checks and query resolution will be performed as outlined in the Data Management Plan. A series of logic and consistency data checks will be conducted to ensure accuracy and completeness of the clinical database. One or more analysis databases, including detailed documentation, will be developed to support the analyses described in this SAP.

8.5 Changes in the Conduct of the Study or Planned Analyses

- Change of the primary analysis population for the primary efficacy endpoint:

The intent to treat population is the primary analysis population for efficacy analyses. It has changed from

‘For efficacy assessments, the intent to treat population (for the primary endpoint; recurrence) will consist of all eyes assigned to Groups 1A and 1B at the start of Study Segment 2, and who receive at least one dose of the study treatment during this segment.’

To

ITT population will consist of all eyes of subjects who enrolled in the study (Segment 1 and Segment 2) and who receive at least one dose of the study treatment.

mITT population will consist of all symptomatic eyes assigned to Groups 1A and 1B at the start of Study Segment 2, who received at least one dose of the study treatment, and had at least one efficacy assessment in Segment 2.

Per-Protocol population will include all eyes included in mITT who have completed both Segment 1 and segment 2 of the study, and have completed at least 80% of the protocol-required doses of the IMP without any major protocol violations or exception that could impact the integrity of study data.

Efficacy analyses will be based on mITT and per-protocol population.

9. Reporting Conventions

Reporting conventions will adhere when possible to the International Conference on Harmonization (ICH) Guidance document E3, “Structure and Content of Clinical Study Reports”. All tables and listings will be in landscape format. All SAS output for tables and listings will be distributed in PDF files.

10. Revision History

SAP Version	SAP Version Date	Rationales for Changes
Version 1	June 18, 2013	N/A
Version 2	June 27, 2014	Update based on Protocol Version 2 Amendment 2 February 2014
Version 3	Aug 28, 2017	Updated to align with the latest protocol version - Protocol Version 2 Amendment 5, November 2016
	Oct 19, 2017	Update after f2f meeting: • Modify analysis population • Add in changes in planned analysis section to reflect the population definition difference in the protocol and in this plan • Added in the new shift tables for comparison of historical data
	May 15, 2018	Update per FDA request dated April 9, 2018
	June 5, 2018	Update per-protocol population definition

Version 4	Aug 24 05, 2020	Update the continuation segment analyses using Protocol Amendment 8 dated April 02, 2020
Version 5	Nov 9, 2020	Update the definition of lack of efficacy and end of study. Update the description of primary and secondary objectives. Added in visit windows

11. References

1. Clinical Study Protocol KB046: A Historically-Controlled Phase II/III study to Evaluate Efficacy and Safety of Kedrion Human Plasminogen Eye Drop Preparation in Patients Diagnosed with Ligneous Conjunctivitis
2. Method for Independent Review and Assessment of Primary and Secondary Efficacy Endpoints
3. Guidance for Industry; E9 Statistical Principles for Clinical Trials – September 1998
4. Guidance for Industry, Providing Regulatory Submissions In Electronic Format - Standardized Study Data (2014)
5. Heidemann DG, Williams GA, Harizer M, Ohanian A, Citron ME. Treatment of ligneous conjunctivitis with topical plasmin and topical plasminogen. Cornea 2003;22(8):760-2.
6. Caputo R. Pucci N, Mori F, Secci J, Novembre E, Frosini R. Long-term efficacy of surgical removal of pseudomembranes in a child with ligneous conjunctivitis treated with plasminogen eyedrops. Thromb Haemost. 2008 Dec;100(6):1196-8.
7. Watts P, Suresh P, Mezer E, et al. Effective treatment of ligneous conjunctivitis with topical plasminogen. Am J Ophthalmol 2002;133:451-5.