# CLINICAL STUDY PROTOCOL

**AMENDMENT 8**
**Issued April 2020**
**of Version 2.0 Amendment 7**
**Date: 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

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| **STUDY SPONSOR** | Kedrion S.p.A.<br>55051 Castelvecchio Pascoli (Lucca)<br>Italy<br>Tel.:<br>Fax: |
| **STUDY MONITORING** | ATLANTIC RESEARCH GROUP, INC., (ARG)<br>2421 Ivy Road, Suite 200,<br>Charlottesville, VA 22903<br>Tel:<br>Fax: |
| **STUDY CODE** | KB046 |
| **SPONSOR'S MEDICAL EXPERT** | <br>Kedrion S.p.A<br>Loc. Il Ciocco 55051 Castelvecchio Pascoli<br>Barga (Lucca) - Italy<br>Tel:<br>Fax:<br>Email: |
| **CO-ORDINATING INVESTIGATOR** | |

# SIGNATURE PAGE

STUDY CODE: KB046
AMENDMENT 8 Issued April 2020
VERSION 2.0, November 2016, Amendment 7

**INVESTIGATOR STATEMENT**

By signing this statement, the investigator declares receipt of a copy of protocol number 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*" and dated *June, April 2020*.

I agree to the conditions as set out in this protocol and fully accept that any change requires prior approval from Kedrion S.p.A.

Additionally, I agree to carry out all terms of this protocol in accordance with International Conference on Harmonization (ICH) Guidelines; the Helsinki Declaration; US Code of Federal Regulations 21 CFR Parts 11, 50, 54, 56, 312 and 314; applicable EU Directives and local regulations.

Finally, I/we will ensure that the investigational medicinal product (IMP) will be used only as described in this protocol.

The information contained in this protocol is provided to me in confidence, for review only by myself, the Institutional Review Board (IRB) or Ethics Committee (EC) authorized to review and approve the study at this study site, and designated research staff participating in this clinical study.

I understand that the information/technology contained in this protocol is proprietary and may not be disclosed to any other party, in any form, without prior authorization from Kedrion S.p.A., except to the extent necessary to obtain informed consent from potential study participants.

_____ _____

Investigator's Name (in block capitals) and signature | Date

Kedrion SpA and ARG commit themselves to satisfying the requirement of the ICH Good Clinical Practice (GCP) Guidelines regarding the responsibilities of the Sponsor, the Code of Federal Regulations 21 CFR Parts 11, 50, 54, 56, 312, 314 and EU Clinical Trial Directives, as applicable.

_____ _____

Study Sponsor's Name (in block capitals) and signature | Date

_____ _____

CRO's Representative (in block capitals) and signature | Date

# INDEX

# STUDY SYNOPSIS

**Sponsor Name:**
Kedrion SpA – Loc. Ai Conti 55051, Castelvecchio Pascoli – Barga (LU)

**Study Title**: 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

| **Product name:** Kedrion Human Plasminogen | **Active Ingredient Name:** Human Plasminogen |
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**Rationale:** To treat Ligneous Conjunctivitis (LC) associated with Type I plasminogen deficiency

**Anticipated Dates of Study:** Approximately May 2013-May 2020

**Individual Subject Participation:**

- **Study Segment 1**
  - 4-week treatment of all subjects (with and without ocular pseudomembranes)
- **Surgery Segment** (if applicable) to remove ocular pseudomembranes (for subjects with remaining pseudomembranes at the end of Segment 1)
  - Up to 2-week continued treatment at Segment 1 dose
- **Study Segment 2**
  - 8-week continued treatment for subjects without ocular pseudomembranes
  - 8-week continued treatment for subjects who have undergone surgery (this segment may be optionally repeated for subjects with partial or total recurrence following surgery)
- **Continuation Segment**
  - Continued treatment for subjects activation of Sponsor Initiated Expanded Access protocol, that will allow the product availability to the patients.
  - Treatment for additional subjects without ocular pseudomembranes activation of Sponsor Initiated Expanded Access protocol, that will allow the product availability to the patients.

**Trial Period:** 12-48 weeks (not including Screening or the Continuation Segment)

**Enrollment Period:** approximately 8 months

**Clinical Phase**: Phase II/III

**Objectives:**

*Primary*

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.
2. Evaluation of the safety of the IMP in symptomatic subjects and asymptomatic subjects with a history of ocular pseudomembranes.

*Secondary*

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

**Study Design:** This will be an open label, historically-controlled, multicenter Phase II/III study.

***Study Segment 1****:* Open label, historically-controlled evaluation of the efficacy and safety of the IMP on symptomatic and asymptomatic subjects diagnosed 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 in subjects diagnosed with LC**.**

***Follow-up in the event of a subject who exits the study****:* Open label evaluation of any change in disease course for all subjects exiting the study for any reason, and no longer treated with IMP.

***Continuation Segment****:* Open label evaluation of safety of the IMP subjects with one or more eyes demonstrating efficacy success (from Groups 1A and 1B),and those who remain asymptomatic (Group 2). Once Groups 1 and 2 are fully enrolled, additional asymptomatic patients (who have not participated in Segments 1 and 2 for efficacy and safety – Maximum 2 subjects) can be entered in the study starting from the continuation segment, after signature of the Informed Consent, assent and data handling authorization (if applicable).

**Efficacy and Safety Evaluation:**

*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, (>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).

*Secondary Efficacy Endpoints*

- Regression of surface areas of existing ligneous pseudomembranes (using objective measurement) at the end of Study Segment 1;
- Time to ligneous pseudomembrane recurrence after surgery or complete regression, (>90%) (days) during Study Segment 2.

*Safety Endpoints*

Clinical safety will be assessed in all subjects receiving any dose of IMP by evaluating:

- Vital signs (blood pressure, heart rate, temperature and respiratory rate.
- Adverse Events (AE - correlation with the IMP assessed as “definitely related” “possibly related,” “probably related” or “not related”) throughout the study.
- Immunogenicity. Blood samples will be taken before starting the treatment at study entry, at the end of Segment 1, every 4 weeks thereafter in Segment 2, every 2 months during continuation segment as well as Termination Visits 1 and 2, to measure antibodies against human plasminogen and antibodies against bovine aprotinin.
- 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.
- Local tolerability. Signs and symptoms of sensitization will be collected throughout the study.

**Study Methodology**: At least ten subjects of any age with LC associated with Type I plasminogen deficiency, for approximately 20 eyes, will be enrolled in the study and treated. A maximum of two additional subjects can be enrolled starting from the continuation segment.

***Study Segment 1***

Depending on the clinical presentation of Subjects’ eyes, each eye will be classified into one of two groups: Group **1** (symptomatic with ocular pseudomembranes) and Group **2** (asymptomatic with no visible, ocular pseudomembrane). Subjects with one eye with pseudomembranes and one eye without will be

classified into Group **1**.

Group **1**: Both eyes will receive 2 drops per eye for 8 times/day of the IMP during waking hours for 4 weeks, even if only one is symptomatic. Subjects will be observed at the end of these 4 weeks, to evaluate pseudomembrane regression at the clinic site.

- Group **1** eyes that achieve complete pseudomembrane regression, (>90%) at the end of the 4 weeks, will be assigned to Group **1A** for continued treatment in Study Segment 2.
- Group **1** eyes that present partial, (>20% and <90%) or no pseudomembrane regression, (<20%) at the end of the 4 weeks, will undergo surgery, within two weeks from the end of Segment 1, to remove the pseudomembranes. These eyes will be then be included in Group **1B** for Study Segment 2 immediately following surgery.

Group **2**: Both asymptomatic eyes at the time of enrollment will receive 2 drops per eye for 6 times/day of the IMP during waking hours for 4 weeks. No more than 5 subjects (10 eyes) will be enrolled in this group.

- Group **2** eyes remaining asymptomatic at the end of the 4 weeks, will continue to receive the IMP at the same dose in Segment 2.
- Group **2** eyes developing pseudomembranes during the 4 weeks, will be transferred to Group **1** upon the first follow-up visit when the pseudomembranes are observed and re-start Study Segment 1, with the dose schedule as per Group 1, if they meet all inclusion/exclusion criteria.

After the first 4 weeks, of treatment in Segment 1, all subjects will undergo a clinical evaluation at the site prior to being entered into Segment 2.

Subjects will be monitored with weekly phone calls and be observed at the end of these 4 weeks to evaluate the safety of the IMP at the clinic when reviewing the Subject Diary data.

***Study Segment 2***

Eyes will be treated for a total of 8 weeks and evaluated for pseudomembrane recurrence. There will be a clinic visit at week 4 of Segment 2 (V2) and a visit to the clinic at week 8 of Segment 2(V3).

**Group 1**

- Group **1A** eyes with complete pseudomembrane regression, (>90%) from Study Segment 1 will receive the IMP at a reduced dose of 2 drops per eye 6 times/day, for 8 weeks in Segment 2.

  If at any time during the 8 weeks of Segment 2 the subject reports recurrence of a pseudomembrane an unscheduled visit will be required and once confirmed by the site, subjects will be re-entered into Group 1 again.

  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 cyle of Segment 1 regression has occurred but recurrence occurs in second repeat of Segment 2 (lower dose), then on the third cycle of Segment 2 the subject **will remain on the higher dose of 2 drops per eye 8 times/day drops for those 8 weeks of Segment 2**. 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, then the subject will be considered a failure and the subject must exit the study. Any time a subject reports pseudomembrane recurrence and unscheduled visit will occur for the site to confirm the recurrence of the pseudomembrane.

  Surgery will not be offered in 1A subjects if the pseudomembranes recur in Segment 2 in the second and third cycles as the recurring pseudomembranes would be new and surgery is not recommended for new lesions.
- Group **1B** eyes undergoing surgery to remove pseudomembranes will receive the IMP at the same dose leading up to their surgery within two weeks of the end of Segment 1, then after the surgery at a descending dose for 8 weeks, starting at 2 drops per eye for 12 times/day for the first week after surgery. The dose will be decreased to 2 drops per eye for 8 times/day over the next 3 weeks and finally, reduced to the maintenance dose of 2 drops per eye for times/day for 4 weeks. Group 1B subjects will have a mandatory Post Surgery Visit at 2 weeks following the surgery, for the assessment

of the pseudomembrane.

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). No second surgery will be performed.

**Group 2**

- If Group **2** eyes remained asymptomatic, subjects will continue to receive the IMP at the same dose as that in Segment 1 for another 8 weeks.
- If a Group **2** eye develops pseudomembrane recurrence at any time during Segment 2, an unscheduled visit will be required and once confirmed by the site, the subject will be transferred to Group **1** and re-start Study Segment 1 if the subject meets all inclusion/exclusion criteria.

***Follow-up After Study Exit***

All subjects exiting the study during Segments 1 and 2 or afterwards, regardless of the reason for the exit, will be asked to consent in writing to be followed for 6 months after study exit. If they consent, they will be given a Subject diary to record any change in disease course, including relapses, spontaneous regressions, surgeries, medications, and other changes in clinical status. During the 6 months follow up period, subjects will not be treated with the IMP. At the end of 6 months, each subject will return to the study site, submit the diary to study staff, and undergo complete clinical assessments on disease status and safety.

***Continuation Segment***

Following enrollment of Groups 1 and 2, additional asymptomatic patients (who have not participated in Segments 1 and 2) can be enrolled in the study starting from the continuation segment.

Subjects with one or more eyes demonstrating efficacy success (from Groups **1A (cycles 1 and 2),** and **1B**) and those who remain asymptomatic (Group **2**) at the end of Segment 2 including the additional asymptomatic subjects (maximum 2 subjects) enrolled in the continuation phase, will be followed until the activation of Sponsor Initiated Expanded Access protocol, that will allow the product availability to the patients. During the Continuation Segment they will continue to receive IMP (dosage regimen 2 drops per eye 4-6 times/day. For Subjects with one or more eyes demonstrating efficacy in Group 1A Cycle 3, who demonstrate no recurrence with the higher maintenance dose in Segment 2, the dose will remain in the Continuation Segment as 2 drops per eye 8 times/day.

Additional clinic visits will be scheduled every 6 months from the start of the continuation segment up to activation of Sponsor Initiated Expanded Access protocol, that will allow the product availability to the patients for safety follow up. A subject Diary will be provided to record any change in the subject's health status. At each clinic visit the Subject Diary will be reviewed and collected by the investigator and a new Diary provided to the subjects. Phone calls will be performed monthly after each clinic visit to remind the subjects to complete the Diary, ask general questions, non leading questions about how they feel and ensure there are no issues with the IMP supplies or home freezer. During this phone call, specific questions on safety will be addressed to document any possible adverse event observed.

Blood samples will be taken every 2 months during the continuation segment for immunogenicity evaluation.

At each time point and starting from the approval of Amendment 4 of this clinical protocol, an extra blood sample must be obtained and stored for possible additional immunogenicity investigation.

During Continuation Segment, a Home Health Agency may be utilized to collect the samples outside of the investigational site. The use of the Home Health Agency is optional. Subjects that opt to use the Home Health Agency must consent prior to initiation of the use of the Home Health Agency for that subject.

By the time of activation of Sponsor Initiated Expanded Access protocol all subjects will be assessed and

data will be reviewed for safety and a better understanding of the disease course.

**Number of Subjects Planned:** At least 10 subjects (for approximately of 20 eyes evaluated). Enrollment in Group 2 will not exceed 5 subjects (10 eyes). A maximum of two additional subjects can be enrolled starting from continuation segment.

Each eye treated will be analyzed separately in the local tolerability and efficacy evaluations in order to increase the sample size.

**Subject Characteristics and Inclusion/Exclusion Criteria:**

***Inclusion Criteria***

Subjects must meet the following criteria for study enrollment:

1. Subjects must be diagnosed with LC associated with Type I plasminogen deficiency, confirmed by the central laboratory and documented at pre-enrollment screening (see Section 5.6.1).
   The concomitant presence of other ligneous pseudomembranes at different sites will not constitute an exclusion criterion.
2. Subjects should have documented historical records of disease course available for a period of at least 6 months surrounding an episode of LC, even if asymptomatic in the past for a newly diagnosed subject, including but not limited to age of LC onset, diagnosis of Plasminogen 1 deficiency, history of pseudomembrane lesions, disease duration, past treatment for LC, response to treatment and/or surgery (including regression and recurrence), before study entrance. If more history than 6 months surrounding an LC episode is available it will be included.
3. Subjects, or their legally authorized representative, in the case of study participants < 18 years of age, should have been informed of the nature of the study, agreed to its provision, signed and dated the informed consent approved by the IRB or EC.
4. Subjects must be available for the duration of the study upon entry.
   The Investigator will make sure that there is no plan for the subject to leave the area of the study site before the end of the study period. If they come from another center, they must agree to be compliant with the protocol mandated study visits and return for follow-up.

**Note**: Additional 2 patients enrolled starting from Continuation segment should be asymptomatic

***Exclusion Criteria***

The following subjects will not be eligible for enrollment:

1. Subjects presenting LC not associated with Type 1 plasminogen deficiency.
2. Subjects with no history of LC lesions for Group 2, for Group 1 the entry lesions could be the first and included as history.
3. Subject presenting antibodies against plasminogen at screening.
4. Subjects with any condition which, in the opinion of the Investigator, might interfere with the evaluation of the study objectives, or participation in this trial.
5. Subjects unwilling to give written informed consent or assent to participation.
6. Subjects who have participated in another clinical trial within 1 month before study initiation, i.e. they have received any test drug within 30 days prior the study.
7. Females of childbearing potential who are either pregnant or not using an adequate method of birth control (adequate is defined as hormonal contraceptive or partner vasectomy for at least 3 months, condoms, intrauterine device [IUD], abstinence or other prescribed birth control).
8. Females who are breastfeeding.
9. Subjects being treated with FFP or Laboratory Grade Plasminogen who have not undergone a washout period of at least 15 days before being considered for this study. This information will be disseminated to subjects ahead of the Screening Visit and will only occur after the signing of an Informed Consent.

**IMP, Dosage and Administration:** Kedrion Human Plasminogen is a sterile human plasma derived plasminogen preparation in the pharmaceutical form of an eye drop for topical ocular use. IMP is presented as a frozen solution, supplied in a vial of neutral clear glass containing 1 ml of a 1 mg/ml sterile solution of protein of which at least 93% is plasminogen.

**Route of Administration:** Topical ocular application

**Dosage regimen:**

The given dose varies based on the symptoms presented:

- Study Segment 1 (Group **1**, pseudomembranes): 2 drops per eye, 8 times/day
- Study Segment 1 (Group **2**, no pseudomembranes): 2 drops per eye, 6 times/day
- Study Segment 2 (Group **1A**, no surgery follow-up, the first two cycles): 2 drops per eye, 6 times/day
- Study Segment 2 (Group 1A, no surgery follow-up, the third cycle): 2 drops per eye, 8 times/day
- Study Segment 2 (Group **1B**, post-surgery follow-up): descending dose regimen - 2 drops per eye, 12 times/day for one week; 2 drops per eye, 8 times/day for three weeks; and 2 drops per eye, 6 times/day for four weeks
- Continuation Segment: (Groups 1A (Cycle 1 and 2), 1B Group 2 and additional subjects enrolled starting from the continuation segment: 2 drops per eye, 4 to 6 times/day (Group 1A Cycle 3): 2 drops per eye, 8 times/day

**Duration of Treatment**:

***Study Segment 1*:**

**Group 1**: 4 weeks. Maybe repeated in Group 1A if pseudomembranes recur at any time during Segment 2. Group 2 subjects will be entered into Group 1 if pseudomembranes occur at any time during Segment 1 and/or up to the end of Segment 2.

**Group 2:** 4 weeks.

***Study Segment 2:***

**Group 1A:** 8 weeks. Maybe repeated at any time up to and including the last week of Segment 2, but the subject must first repeat Segment 1 for Group 1. This can occur up to three times, On the third time the dose will remain at 2 drops 8 times a day.

**Group 1B:** Upto 2 weeks for the surgery to be performed. 8 weeks for Segment 2. May be repeated if pseudomembrane recurrence occurs > 2 weeks after surgery.

**Group 2:** 8 weeks.

***Note:*** *Surgery*: maximum 2 weeks. Upto the time of surgery they will continue the dose schedule as per Group 1 Segment 1.

***Continuation Segment*:** Until activation of Sponsor Initiated Expanded Access protocol, that will allow the product availability to the patients.

***Follow-up After Study Exit*:**

Duration of the Follow Up Period for Subjects exiting the study during Segment 1 and 2 or afterwards, regardless of the reasons for the exit (Follow Up after study exit) is 6 months.

**Criteria for Evaluation:**

*Efficacy*

The primary efficacy endpoints in this study will be measured by the number of eyes that develop recurrent pseudomembranes in Segment 2 after initially showing total regression after treatment or after surgical

excision.

Treatment with the IMP will be considered effective if the rate of pseudomembrane recurrence is lower than the recurrences in the subjects' historical data, with each evaluated eye compared with its own history of recurrence. Efficacy outcomes will be classified into 3 categories: complete success (no recurrence by the end of Segment 2), partial success (recurrence observed 2 weeks or more after 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 (recurrence within 2 weeks of the start of Segment 2 or if at repeat cycles of Segment 1 for Group 1A, the pseudomembranes do not regress after Segment 1).

Secondary efficacy will be measured by the size of pseudomembrane regression:

1. Percentage decrease in the objective measurement of pseudomembrane surface area from baseline to the end of Segment 1.
   The objective measurement will be performed by taking scaled photographs of the eyes and comparing the pictures taken at study visits. as detailed in the study protocol. The photographs will be evaluated by at least 2 ophthalmologists using a [redacted]
2. Proportion of eyes with a reduction of the overall membrane surface area as measured with the method above at the end of Segment 1.
3. Time to pseudomembrane recurrence after complete regression or surgery will be measured in days.

*Safety*

1. Percentage of subjects who develop antibodies against human plasminogen.
2. Percentage of subjects who develop antibodies against bovine aprotinin.
3. Percentage of subjects who experience signs and symptoms of sensitization.
4. Percentage of subjects who experience AE (correlation with the IMP assessed as possible, probable, or definite).

**Statistical Methods:** This study will use descriptive statistics to describe the study observations and analyze trends. The study will enroll, treat and evaluate at least 10 subjects to provide data for approximately of 20 evaluated eyes. Each subject will serve as his or her 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. The sample size is not supported by any statistical power calculations, as the low prevalence of this disease makes a large sample size difficult to attain.

Efficacy responses will be descriptively presented on the basis of individual eyes along with historical clinical data. Time to recurrence will be presented using a Kaplan-Meier survival plot. AE and other safety parameters will be presented on a per subject basis.

# 1 LIST OF ABBREVIATION AND DEFINITIONS OF TERMS

| | |
|---|---|
| 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 |
| EC | Ethics Committee |
| eCRF | Electronic Case Report Form |
| 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 |
| IUD | Intrauterine Device |
| IMP | Investigational Medicinal Product |
| IND | Investigational New Drug |
| IRB | Institutional Review Board |
| Kg | Kilogram |
| LC | Ligneous Conjunctivitis |
| MedDRA | Medical Dictionary for Regulatory Activities |
| 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 |

# 2 ETHICS

## 2.1 Institutional Review Board (IRB)

ICH Topic E6 Guidelines for GCP requires that an IRB oversee all investigational drug studies. This board or committee will approve all aspects of the study, including the protocol, written Informed Consent Form (ICF) and Consent Form for Genetic Test and any subject information sheets to be used, prior to initiation of the study. The Investigator will provide the Sponsor with a copy of the communication from the committee to the Investigator indicating approval of the protocol and consent form/information sheets. All amendments to the protocol must be reviewed and approved prior to implementation, except where necessary to eliminate apparent immediate hazards to human subjects.

The Investigator will be responsible for obtaining annual IRB renewal and submitting serious adverse event (SAE) reports to the IRB for the duration of the study (per IRB policies and procedures). Copies of the Investigator's report and/or copies of the IRB extension approval must be sent to the Sponsor and/or designee.

The study protocol and amendments, informed consent documents and subject recruitment documents, protocol deviations and violations will be submitted to the IRB according to the requirements of each of these institutions.

## 2.2 Ethics Committee (EC)

In Europe (EU), the trial will not start until written favorable opinion is received from the EC and no objection is raised by the Competent Authority (CA).

## 2.3 Study Conduct

This study will be conducted in compliance with the United States (US) Food and Drug Administration (FDA) regulations and guidelines, ICH Guideline for GCP E6 (R1), 2002, European Medicinal Agency (EMA) regulations and guidelines (see Section 18) and principles of the Declaration of Helsinki (see Section 19).

## 2.4 Informed Consent and Assent

In compliance with the recommendations of the Declaration of Helsinki, each Subject must be adequately informed of the aims, methods, anticipated benefits, potential hazards, and the discomfort the study may entail, as well as the subject's right to abstain from participation in the study and to withdraw at any time.

No subject can be entered into the study before his/her informed consent or assent has been obtained. The Investigator will not undertake any investigation specifically required only for the clinical study until valid consent has been obtained. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the Investigator must obtain that assent, in addition to the consent of the legally authorized representative.

Subjects will be permitted to take the Informed Consent Form (ICF) home and talk it over with others before they sign it. The terms of the consent and the date and time of day when it was obtained must be documented in the Case Report Form (eCRF). The ICF, with the date and time of day when it was signed, must be retained by the Investigator as part of the study

records. A copy of the signed ICF will be given to the subject or subject's legally authorized representative.

An additional Consent Form for Genetic Test will be collected. There will also be a separate ICF and/or assent for additional subjects entering only into the Continuation Segment.

If a subject refuses the Genetic Test, the Subject can still participate in the study by signing the ICF. The ICF and Consent Form for Genetic Test must be submitted by the Investigator with the protocol to the IRB or EC for approval. ARG/Kedrion S.p.A. supplies the proposed consent forms, which are considered appropriate for the study. Any changes suggested to the proposed ICF and Consent Form for Genetic Test by the Investigator must be agreed to by ARG/Kedrion S.p.A. before submission to the IRB or EC and a copy of the approved version must be provided to the monitor.

If a subject refuses the safety follow up for 6 months once exited from the study, then follow up will cease once they exit and conduct a termination visit if possible.

Should a protocol amendment be required, the ICF and Consent Form for Genetic Test may be revised to reflect the changes of the protocol. If the consent forms are revised, it is the Investigator's responsibility to ensure that the amended consent forms are reviewed and approved by the IRB or EC and that these amended forms are signed by all subjects subsequently entered in the study as well as those currently in the study.

### 2.5 Confidentiality

The Investigator will ensure that the subjects' anonymity will be maintained. The privacy rules of the US Health Insurance Portability and Accountability Act (HIPAA) will be followed to obtain authorization for most uses and disclosures of Protected Health Information. On eCRFs or other documents submitted to the Sponsor or its designee, subjects will not be identified by their names, but by an identification code, consisting of the combination of subject's initials and study number. Documents not for submission to Kedrion S.p.A. or its designee (e.g., the site confidential subject enrollment log and original subjects' consent forms) will be maintained by the Investigator in strict confidence.

# 3 INVESTIGATOR AND STUDY ADMINISTRATIVE STRUCTURE

Multi-center, open-label, phase II/III clinical trial coordinated by [redacted]

Principal Investigator (PI): [redacted]

Number of Centers: 3. Two EU sites and one US site.
CRO Representative: Atlantic Research Group, 2421 Ivy Road, Suite 200, Charlottesville, VA 22903

The Vendors List is reported in Appendix 2 (§19.2).

# 4 INTRODUCTION AND RATIONALE

LC is a rare form of chronic “idiopathic membranous” conjunctivitis, caused by a plasminogen deficiency. The disease is characterized by initial chronic tearing and redness of the conjunctivae followed by the formation of pseudomembranes, mostly on the palpebral surface. LC was first described in 1847 by Bouisson and the term ‘Ligneous Conjunctivitis’ was introduced by Borel in 1933.

The characteristic pseudomembranes are amorphous, hyaline-like and have been shown to contain mainly clotted fibrin(ogen), along with other plasma proteins such as albumin and immunoglobulins (mainly IgG) and eosinophilic material (Schuster & Seregard, 2003). As these pseudomembranes grow, they progress to white, yellow-white, or red thick, wood-like masses that replace the normal mucosa. The pseudomembranes can sometimes resemble amyloidosis of the eye, but they are negative for Congo red staining used to distinguish amyloidosis. Most commonly, these firm sessile or pedunculated pseudomembranes appear on the upper tarsal conjunctiva. Involvement of the lower eyelid and the bulbar conjunctiva has been reported less frequently. In addition, many patients exhibit mucoid discharge from affected eyes. Multiple recurrences after local excision have been described.

Since the first case report, various etiological agents have been proposed, including surgical trauma, autoimmune phenomena, hypersensitivity reactions and infectious agents (Schuster et al., 2001). Schott showed that plasminogen, a key element of fibrinolysis, was undetectable in conjunctival tissue (Schott et al., 1998). Plasmin plays an important role in intra- and extra vascular fibrinolysis and wound healing, because it contributes to the displacement of fibrin-rich extra cellular matrix in healing skin. Furthermore, plasmin facilitates keratinocyte division, migration, and differentiation, and supports closure of skin wounds. The central feature of LC is an impaired wound-healing capacity with an arrest at the stage of granulation tissue formation and excessive fibrin deposition.

Pseudomembranous disease associated with plasminogen deficiency is typically an autosomal recessive disorder that results from a homozygous or compound heterozygous defect (Schuster & Seregard, 2003). Since 1997, Schuster demonstrated distinct homozygous and compound heterozygous mutations; in the plasminogen gene to be common in patients with LC and clearly confirmed autosomal recessive inheritance of these disorders (Schott et al., 1998; Schuster, et al., 2001; Schuster et al., 1997; Schuster et al., 1999). Several genetic defects have been documented that lead to plasminogen deficiency (Schuster et al., 1997; Schuster et al., 2001; Siboni et al., 2007). Tefs et al. identified a lysine replaced by a glutamine at codon 19 (K19E mutation) (Tefs et al., 2006), which was also documented in other reports (Siboni et al., 2007). This mutation was evaluated in the Scottish blood donor population and was found in 13 of the 15 subjects evaluated with documented decreased plasminogen levels (Tefs et al., 2003). Studies in mice have also been conducted, confirming the link between the plasminogen deficiency and clinical manifestations (Kao et al., 1998; Drew et al., 1998).

Inherited plasminogen deficiency in humans can be divided into two types: true plasminogen deficiency (Type I, or hypoplasminogenemia) and dysplasminogenemia (Type II). In the former, both plasminogen level and functional activity are reduced, while in the latter only the functional plasminogen activity is significantly decreased, but plasminogen level can be normal or only slightly reduced (Tefs et al., 2006).

Infants and children are predominantly affected, but the disease may manifest at any age. In predisposed subjects, LC may be triggered by local injuries, local and systemic infections,

lime burns, and surgical interventions of the eyes (Schuster & Seregard, 2003). LC is chronically debilitating due to the risk of corneal involvement and subsequent blindness.

***Treatment with Human Plasminogen***

Treatment with plasminogen replacement has shown to be effective in reducing pseudomembranes to various extents. Whereas intravenous treatment presents many problems and may be impractical due to the necessity of a central venous catheter for venous access to overcome the issues related to the short half-life of plasminogen, topical treatment allows easy administration apparently with satisfactory results (Schuster & Seregard, 2003; Schott et al., 1998; Watts et al., 2002). The efficacy of a therapy with plasminogen eye drops has been shown (Heidemann et al., 2003). The response was prompt and dramatic resulting in complete resolution of the membranes in one month. The membranes recurred after the drops were discontinued, but again resolved promptly after treatment was restarted.

Results observed in the Heidemann's case have been confirmed by another case that provides additional evidence of efficacy of plasminogen replacement treatment (Caputo et al., 2008). Caputo describes the case of a two-year old boy with Type 1 plasminogen deficiency and LC, in which surgical removal of pseudomembranes was combined with long-term use of topical plasminogen drops. Administering topical plasminogen two months before surgery was successful with a good control of symptoms, though chronic pseudomembranes were not reabsorbed due to severe conjunctival degeneration. Older, chronic pseudomembranes were not responsive to plasminogen treatment and surgery was deemed mandatory to remove them. Following surgery, the patient was maintained on plasminogen eye drops. At a 12-month follow-up after surgery, pseudomembrane recurrence has not been observed in both eyes. Further studies are necessary to confirm the hypothesis which suggests that surgical removal of pseudomembranes can be safe and effective in the long term if it is associated with the topical administration of plasminogen.

***Kedrion Human Plasminogen (IMP)***

Human plasminogen has been designated as an Orphan Medicinal Product for the treatment of LC with Commission Decision EU/3/07/461 dated 03 August 2007. On 07 June 2010 the FDA also granted Human plasminogen the Orphan Drug Designation for the treatment of LC.

Kedrion Human Plasminogen 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.

# 5 STUDY OBJECTIVES

This study will examine the efficacy and safety of a plasminogen eye drop solution for the treatment of LC. The objectives of this study are:

*Primary Objectives:*

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.
2. Evaluation of the safety of the IMP in symptomatic subjects and asymptomatic subjects with a history of ocular pseudomembranes.

*Secondary Objectives:*

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

# 6 INVESTIGATIONAL PLAN

## 6.1 Overall Study Design and Plan Description

This will be a multicenter, open-label study. At least ten subjects (for approximately of 20 eyes, as some Subjects may not have both eyes with lesions) of any age with LC associated with Type I plasminogen deficiency will be enrolled in the study and treated.

**Study Segment 1**

Depending on the clinical presentation of Subjects' eyes, each eye will be classified into one of two groups: Group **1** (symptomatic with ocular pseudomembranes) and Group **2** (asymptomatic with no visible, ocular pseudomembrane). Subjects with one eye with pseudomembranes and one eye without will be classified into Group **1**.

Group **1**: Both eyes will receive 2 drops per eye 8 times/day of the IMP during waking hours for 4 weeks, even if only one eye is symptomatic. Subjects will be observed at the end of these 4 weeks to evaluate pseudomembrane regression at the clinic site.

- Group **1** eyes that achieve complete pseudomembrane regression, (>90%) at the end of 4 weeks will be assigned to Group **1A** for continued treatment in Study Segment 2.
- Group **1** eyes that present partial, (>20% but <90%) or no pseudomembrane regression, (<20%) at the end of 4 weeks will undergo surgery, within 2 weeks of the end of Segment 1, to remove the pseudomembranes. These eyes will be then be included in Group **1B** for Study Segment 2 immediately following surgery.

Group **2**: Both asymptomatic eyes at the time of enrollment will receive 2 drops per eye for 6 times/day of the IMP during waking hours for 4 weeks. Enrollment in Group 2 will be approximately of 5 subjects (10 eyes). Subjects will be monitored with weekly phone calls and be observed at the end of these 4 weeks to evaluate the safety of the IMP at the clinic when reviewing the Subject Diary data.

- Group **2** eyes remaining asymptomatic at the end of Study Segment 1 will continue to receive the IMP at the same dose in Segment 2.
- Group **2** eyes developing pseudomembranes during Study Segment 1 will be transferred to Group **1** at the first evaluation visit when the pseudomembranes are

observed and re-start Study Segment 1, with the dose schedule of Group 1, if they meet all inclusion/exclusion criteria.

After the 4 weeks of treatment in Segment 1, all subjects will undergo a clinical evaluation at the site prior to being entered into Segment 2.

Subjects will be monitored with weekly phone calls and be observed at the end of these 4 weeks to evaluate the safety of the IMP at the clinic when reviewing the Subject Diary data.

**Study Segment 2**

Eyes will be treated for a total of 8 weeks and evaluated for pseudomembrane recurrence. There will be a clinic visit at week 4 of Segment 2 (V2) and a visit to the clinic at week 8 of Segment 2(V3). Group **1**

- Group **1A** eyes with complete pseudomembrane regression, (>90%) from Study Segment 1 will receive the IMP at a reduced dose of 2 drops per eye for 6 times/day for 8 weeks in Segment 2.
  If at any time during 8 weeks of Segment 2 the subject reports recurrence of a pseudomembrane, an unscheduled visit will be required and once confirmed by the site, subjects will be re-entered into Group 1 again.
  Subjects can recycle through Group 1 and 1A twice only. 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 Segment 1 regression has occurred but recurrence occurs in second repeat of Segment 2 (lower dose), then on the third cycle of Segment 2 the subject **will remain on the higher dose of 2 drops per eye 8 times/day for those 8 weeks of Segment 2**. 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, then the subject will be considered a failure. Any time a subject reports pseudomembrane recurrence and unscheduled visit will occur for the site to confirm the recurrence of the pseudomembrane.
  Surgery will not be offered in 1A subjects if the pseudomembranes recur in Segment 2 in the second and third cycles as the recurring pseudomembranes would be new and surgery is not recommended for new lesions.

- Group **1B** eyes undergoing surgery to remove pseudomembranes will receive the IMP at the same dose leading upto their surgery within two weeks of the end of Segment 1, then after the surgery at a descending dose for 8 weeks, starting at 2 drops per eye for 12 times/ day for the first week after surgery. The dose will be decreased to 2 drops per eye for 8 times/day over the next 3 weeks and finally, reduced to the maintenance dose of 2 drops per eye for 6 times/day for 4 weeks. Group 1B subjects will have a mandatory Post Surgery Visit at 2 weeks following surgery, for the assessment of pseudomembranes.
  - *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 failure and exit the study. Subjects with an eye(s) developing pseudomembrane recurrence more than 2 weeks after surgery will be considered as a partial success and given the option to repeat the IMP*

*treatment regimen of Study Segment 2 (descending dose regimen). No second surgery will be performed.*

Group **2**

- If Group **2** eyes remain asymptomatic, they will continue to receive the IMP at the same dose as that in Segment 1 for another 8 weeks.

If a Group **2** eye develops pseudomembrane recurrence at any time during Segment 2, an unscheduled visit will be required and once confirmed by the site, the subject will be transferred to Group **1** and re-start Study Segment 1 if the subject meets all inclusion/exclusion criteria.

Primary efficacy evaluations will take place at the end of Study Segment 2. Safety evaluations will take place throughout the study during Segments 1, 2 and during the continuation segment (if applicable).

During Segment 1 and 2 the site will call the subject weekly to remind them to complete the Subject Diary, ask general questions non leading questions about how they feel and ensure there are no issue with the IMP supplies or home freezer.

### Follow-up After Study Exit

All subjects exiting the study during Segments 1 and 2 or afterwards, regardless of the reason for the exit, will be asked to consent in writing to be followed for 6 months after study exit. If they consent they will be given a Subject diary to record any change in disease course, including relapses, spontaneous regressions, surgeries, medications, and other changes in clinical status. During the 6 months follow up period subjects will not be treated with the IMP. At the end of 6 months, each subject will return to the study site, submit the diary to study staff, and undergo complete clinical assessments on disease status and safety.

### Continuation Segment

Two additional asymptomatic patients (who have not participated in Segments 1 and 2) can be enrolled in the study starting from the continuation segment, only after Groups 1 and 2 are fully enrolled.

Subjects with one or more eyes demonstrating efficacy success (from Groups **1A**, (cycles 1 and 2) and **1B**), and those who remain asymptomatic (Group **2**) at the end of Segment 2 including the additional asymptomatic subjects enrolled in the continuation phase will be followed until activation of Sponsor Initiated Expanded Access protocol. During the Continuation Segment they will continue to receive IMP (dosage regimen 2 drops/eye 4-6 times/day) by the time of activation of Sponsor Initiated Expanded Access protocol. For Subjects with one or more eyes demonstrating efficacy in Group 1A Cycle 3, who demonstrate no recurrence with the higher maintenance dose in Segment 2, the dose will remain in the Continuation Segment as 2 drops per eye 8 times/day. All subjects will be observed and data will be reviewed for safety and a better understanding of the disease course.

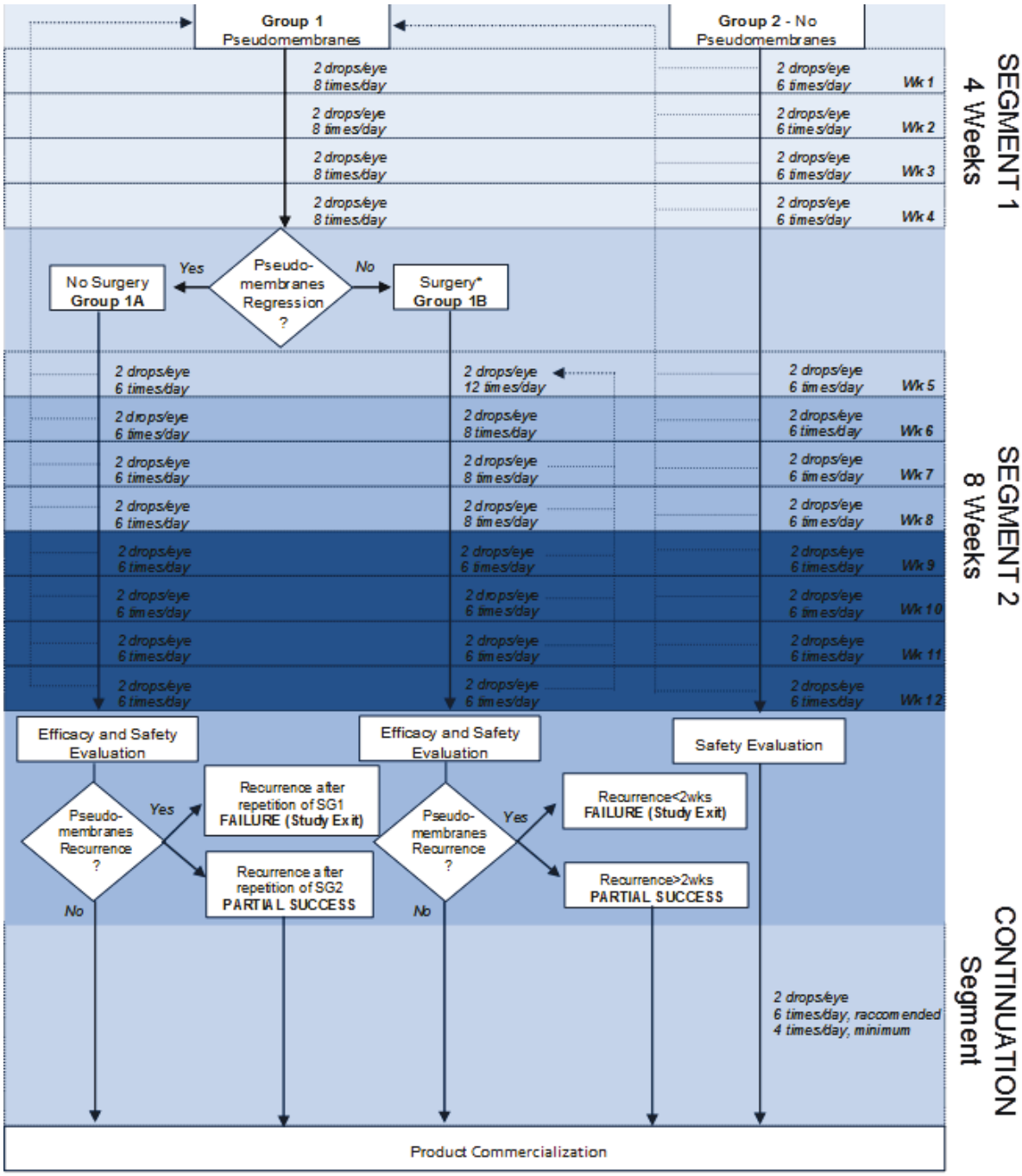
Additional clinic visits will be scheduled every 6 months from the start of the continuation segment up to activation of Sponsor Initiated Expanded Access protocol for safety follow up. A subject Diary will be provided to record any change in the subject's health status. A each clinic visit the Subject Diary will be reviewed and collected by the investigator and a new Diary provided to the subjects.

Blood samples will be taken every 2 months during the continuation segment for immunogenicity evaluation.

During Continuation Segment, a Home Health Agency may be utilized to collect the samples outside of the investigational site. The use of the Home Health Agency is optional. Subjects that opt to use the Home Health Agency must consent prior to initiation of the use of the Home Health Agency for that subject.

At each time point and starting from the approval of Amendment 4 of this clinical protocol, an extra blood sample must be obtained and stored for possible additional immunogenicity investigation. The site will call the subject monthly to remind them to complete the Subject Diary, ask general questions non leading questions about how they feel and ensure there are no issues with the IMP supplies or home freezer.

**Figure 1: Overall Study Design**




** Up to a maximum of 2 weeks - pre-operative dose 2 drops/eye - 8 times/day*

***NOTE**: Subject exiting the study during Segment 1 and 2 or immediately afterwards, regardless of the reasons for the exit, will be asked to consent in writing to be followed for 6 months after study exit (last dose).*

Subjects in 1A after two cycles at the reduced dose in Segment 2, will have the dose increased to 2 drops 8 times/day in cycle 3 of Segment 2.

### 6.1.1 Efficacy Assessments

The efficacy of plasminogen will be assessed in Study Segments 1 and 2. Only Group **1** eyes will be assessed for efficacy. During Study Segment 2, subjects will be closely observed for the recurrence of pseudomembranes after surgery or regression. All pseudomembranes will be counted and the surface areas measured over time.

***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 (>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 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 pseudomembranes do not regress after Segment 1.

The clinical course of each eye during the study, including pseudomembranes regression and recurrence, will be compared with each 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 pseudomembranes recurs, it will be treated as a treatment failure.

***Secondary Efficacy Endpoints***

- Regression in surface areas of existing ligneous pseudomembranes (using objective measurement) at the end of Study Segment 1;
- Time to ligneous pseudomembrane recurrence 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 are [redacted]

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.

### 6.1.2 Safety Assessments

Clinical safety will be assessed in all subjects (Groups **1** and **2** and the additional subjects in the continuation phase) receiving any dose of the IMP by evaluating:

- Vital signs, including blood pressure, heart rate, temperature, respiratory rate.
- AEs will be collected from receiving the first dose and throughout the study, including the continuation phase, up to activation of Sponsor Initiated Expanded Access protocol. 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).
- AEs will be collected up to 30 days post last dose of IMP.
- Immunogenicity. 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, every 4 weeks thereafter in Segment 2 and every 2 months during continuation segment to measure the following markers: 1) antibodies against human plasminogen and 2) antibodies against bovine aprotinin.
- 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.
- Local tolerability. Signs and symptoms of sensitization will be collected throughout the study.

## 6.2 Study Duration

The planned study duration is as follows:

**Table 1: Study Duration**

| Activity | Time frame |
|---|---|
| **Subject enrollment interval** | Continual |
| **Duration of individual subject's participation, excluding 4 weeks in the screening phase.** | Minimum: 12 weeks + continuation segment;<br>Maximum: 48 weeks + continuation segment |
| **Duration of treatment** | Study Segment 1: 4 weeks (may be repeated for Group **2**)<br>Surgery preparation and surgery (if necessary): 2 weeks<br>Study Segment 2: 8 weeks (may be repeated for Group **1B**) |
| **Continuation segment** | Until activation of Sponsor Initiated Expanded Access protocol |
| **Total duration of study*** | 12-48 weeks (plus continuation segment) |

* Subjects exiting the study during Segment 1 and 2 or afterwards, regardless of the reasons for the exit will be followed up for 6 months after study exit.

The above timelines may be subjected to change in relation to accrual time.

The maximum study duration is estimated at 48 weeks for Segments 1 and 2. For the Continuation Segment the duration will depend on the time to activation of Sponsor Initiated Expanded Access protocol . Timing of study segments will vary and flexibility is allowed for surgery scheduling and/or recycling through Groups and dependent on outcomes of regression and/or recurrence. Therefore, study weeks will not be listed consecutively but will be coded and assigned as subject hits study milestones.

| Study segment | Corresponding Week Designation |
|---|---|
| **Study Segment 1** | Week S1-1 through Week S1-4 (may be repeated) |
| **Surgery Window** | Week SW-1 through SW-2 |
| **Study Segment 2** | Week S2-1 through Week S2-8 (may be repeated) |
| **Continuation segment** | Week C-1 through up to activation of Sponsor Initiated Expanded Access protocol |

Subjects exiting the study during Segment 1 and 2 or afterwards, regardless of the reasons for the exit will be followed up for 6 months from the Week they exit.

Additional clinic visits will be scheduled every 6 months from the start of continuation segment up to activation of Sponsor Initiated Expanded Access protocol to submit their Subject diaries and receive a safety follow-up visit for clinical assessments of disease status and safety and a physical examination.

During the Continuation Segment, blood samples will be taken every 2 months for immunogenicity evaluation.

### 6.3 Discussion of Study Design

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 and asymptomatic 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.

**Follow-up in the event of a subject who exits the study:** Open label evaluation of any change in disease course for all subjects exiting the study for any reason, and no longer treated with IMP.

**Continuation Segment:** Evaluation of safety of the IMP subjects with one or more eyes demonstrating efficacy success (from Groups 1A and 1B), and of those who remain asymptomatic (Group 2) including the additional subjects enrolling in the continuation segment only.

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 Subjects and a lack of approved treatment preclude the use of a randomized, controlled design.

### 6.4 Selection of Study Population

The study population of this Phase II/III trial will consist of at least 10 subjects with LC associated with Type I plasminogen deficiency, for approximately of 20 affected eyes. Enrollment in Group 2 (asymptomatic), will be not exceed 5 subjects (10 eyes). A maximum of two additional subjects can be enrolled starting from continuation segment, only after Groups 1 and 2 are fully enrolled.

Due to the small number of LC Subjects, accrual time may become a critical factor, so a multicenter study is planned. Subjects will be recruited from hospitals and referral centers across the US and EU. Efforts will be made to recruit subjects through physicians who treat cases of this rare disease.

**Inclusion Criteria** Subjects must meet the following criteria for study enrollment:

1. Subjects must be diagnosed with LC associated with Type I plasminogen deficiency, confirmed by the central laboratory and documented at pre-enrollment screening (see Section 6.6.1).
   The concomitant presence of other ligneous pseudomembranes at different sites will not constitute an exclusion criterion.
2. Subjects should have documented historical records of disease course available for a period of at least 6 months surrounding an episode of LC, even if asymptomatic in the past for a newly diagnosed subject, including but not limited to age of LC onset, diagnosis of Plasminogen 1 deficiency, history of pseudomembrane lesions, disease duration, past treatment for LC, response to treatment and/or surgery (including regression and recurrence), before study entrance. If more history than 6 months surrounding an LC episode is available it will be included
3. Subjects, or their legally authorized representative, in the case of study participants < 18 years of age, should have been informed of the nature of the study, agreed to its provision, signed and dated the informed consent approved by the IRB or IEC.
4. Subjects must be available for the duration of the study upon entry.
   The Investigator will make sure that there is no plan for the subject to leave the area of the study site before the end of the study period. If they come from another center, they must agree to be compliant with the protocol mandated study visits and return for follow-up.

Note: The two additional patients enrolled starting from Continuation segment should be asymptomatic.

**Exclusion Criteria** The following subjects will not be eligible for enrollment:

1. Subjects presenting LC not associated with Type 1 plasminogen deficiency.
2. Subjects with no history of LC lesions for Group 2, for Group 1 the entry lesions could be the first and included as history.
3. Subject presenting antibodies against plasminogen at screening.
4. Subjects with any condition which, in the opinion of the Investigator, might interfere with the evaluation of the study objectives, or participation in this trial.

5. Subjects unwilling to give written informed consent or assent to participation.
6. Subjects who have participated in another clinical trial within 1 month before study initiation, i.e. they have received any test drug within 30 days prior the study.
7. Females of childbearing potential who are either pregnant or not using an adequate method of birth control (adequate is defined as hormonal contraceptive or partner vasectomy for at least 3 months, condoms, intrauterine device [IUD], abstinence or other prescribed birth control).
8. Females who are breastfeeding.
9. Subjects being treated with FFP or Laboratory Grade Plasminogen who have not undergone a washout period of at least 15 days before being considered for this study. This information will be disseminated to subjects ahead of the Screening Visit and will only occur after the signing of an Informed Consent.

### 6.4.1 Removal of Subjects from Treatment or Assessment

Subjects or legal guardians may withdraw their consent for participation in the study at any time without prejudice. Additionally, the Investigator may withdraw a subject if, in his/her clinical judgment, it is in the best interest of the subject or if the subject cannot comply with the protocol. Wherever possible, the tests and evaluations listed for the termination visit should be carried out. The Sponsor should be notified of all study withdrawals within 24 hours.

### 6.4.2 Replacement policy

Additional subjects may be recruited during the enrollment study period to ensure that total number of subjects in the study may be at least 10. Subjects who have withdrawn from the ongoing study will not be replaced.

## 6.5 Investigational Medicinal Product

### 6.5.1 Plasminogen

Kedrion Human Plasminogen (the IMP) 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.

The preparation is virus inactivated by a solvent/detergent treatment during the manufacturing process. To ensure the removal of very small viruses (such as parvovirus B19) and tissue spongiform encephalitis (TSE) agents, a nanofiltration step has been added to the manufacturing process as a further step of viral removal through a 15nm (or 20nm) pore size membranes.

During processing, the plasma pool is supplemented with bovine aprotinin to prevent the conversion of plasminogen to plasmin. The plasminogen concentration (1mg/ml) is determined using the Bradford method.

**Route of administration**

Topical ocular application.

**Dose regimen**

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 | 2 drops | 6 times |
| | Group 1A (no surgery) 3rd cycle | 2 drops | 8 times |
| | Group 1B (post-surgery) | 2 drops | Descending frequency:<br>12 times (Week S2-1)<br>8 times (Week S2-2 to S2-4)<br>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<br>Group 2 (no pseudomembrane)<br>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 modified from published clinical data (Watts, et al., 2002) and the clinical experience in a single patient in Italy treated with human plasminogen preparation provided by Kedrion S.p.A. (Caputo et al., 2008).

The IMP will be packaged in single-use, and each vial will contain 1 ml of the Human Plasminogen solution in a 10ml vial. In the conduct of this study, at least 3 of IMP will be used. IMP will be duly labeled and packed for the clinical trial before delivery to the local investigator and also to the subjects homes from the Central Repository with ancilliary supplies

**Undesirable effects**

Please refer to Investigator's Brochure.

**Instructions for use and handling**

The product must be thawed, brought to room temperature, and visually inspected for particulate matter and discoloration prior to administration.

Additional instructions for preparation, administration use and storage will be provided in the Subject Manual and IMP Manual.

**Method of administration**

Subjects will self-apply IMP by dropping solution into the open eye, directly from a syringe barrel. Detailed instructions are supplied in the Study Manual and in the IMP Manual.

**Shelf life**

The product shelf life will be suitable for the clinical study period. Each lot of IMP will have an expiry date for frozen shelf life. Once thawed, the IMP can be used within [redacted] hours at refrigeration temperatures.

**Special precautions for storage**

The IMP should remain frozen and stored at [redacted] until use. Do not use if product is not frozen upon receipt. The frozen IMP should be thawed at room temperature for 30 minutes before application.

The product is intended for single use and should not be used for more than one administration.

Investigators are responsible for the IMP storage at their study center in compliance with all the necessary requirements.

The participating subjects will be allowed to use the IMP while at home. Subjects will be given strict instructions about the storage and use of the IMP during their home treatment regimen. Study participants will collect data on IMP administrations occurring at home in a Sponsor-issued Subject Diary. As described in the Subject Manual, instructions and supplies are provided to return used and unused vials to the site with the Subject Diaries and the Shipping Receipt Forms to perform drug accountability.

**Label text***

***English Language***

**Kedrion S.p.A.** **Study Code:** KB046

**Number Center -Subject: _____**

**IMP / Potency:** Plasminogen 1mg/ml

**Volume:** 1 ml

**Investigator: _____**

**Pharmaceutical form:** Frozen solution/drops for topical ocular administration

**Route of Administration:** topical ocular

**Dosage:** See study protocol

**Instruction for use:** See study protocol

**Special precautions for storage:** Store [redacted]

**KEEP AWAY FROM CHILDREN**

**CAUTION: NEW DRUG – LIMITED BY FEDERAL LAW TO INVESTIGATIONAL USE**

***Italian Language***

**Kedrion S.p.A.** **Codice Studio:** KB046

**Numero Centro-Soggetto: _____**

**IMP / Potenza:** Plasminogeno 1mg/ml

**Volume:** 1 ml

**Sperimentatore: _____**

**Forma Farmaceutica:** Soluzione congelata/gocce per somministrazione topica oculare

**Via di somministrazione:** topica oculare

**Dosaggio:** Vedere protocollo di Studio Clinico

**Istruzioni per l'uso:** Vedere protocollo di Studio Clinico

**Speciali precauzioni per la conservazione:** Conservare a T [redacted]

**TENERE FUORI DALLA PORTATA DEI BAMBINI**

**USARE ESCLUSIVAMENTE PER STUDIO CLINICO**

Expiry dates of frozen IMP may be on the side of the carton, or supplied as a document to the site who will inform study subjects.

*The first batch of IMP (01LP11), does not have the batch number on the label or carton, and also has hand written identifiers. The sites and subjects will not document each individual number, only that the batch number they belong to is 01LP11.

### 6.5.2 Treatment

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

### 6.5.3 Concomitant medications

Details concerning the use of concomitant medications will be recorded.

Subjects are not permitted to use other plasminogen or plasminogen-containing products (e.g. FFP, laboratory grade plasminogen) during the course of the study. If a subject has been treated with plasminogen-containing products prior to study entry, they must have undergone a 15 day washout period, prior to screening.

Corticosteroids and antibiotics used post-surgery (up to 4 weeks maximum) are considered the standard of care. Other treatments for LC will **not** be allowed during the course of the trial.

## 6.6 Study Procedures

For this study, the Investigator may be an ophthalmologist or a hematologist who is familiar with this disease. If the Investigator is not a surgical ophthalmologist, a surgical ophthalmologist will be recruited as a sub-investigator. An Ocular Manual will be provided to each site and each Investigator and sub-Investigator will receive training for the eye exam and to take photographs of the eye and LC pseudomembranes (if applicable), and to capture images for further evaluation. An eye examination form will be supplied to the ophthalmologists to capture other tests required to assess corneal involvement also.

### 6.6.1 Screening

Prior to the start of the study, eligible subjects will receive the appropriate informed consent form and subject information booklet, if applicable. Subjects will be encouraged by the Investigator to ask questions regarding their participation in the study. Subjects who agree to participate in the study will then sign and date the informed consent. For subjects <18 years of age, the parents or legal guardian must also sign and date the written informed consent.

Subjects will report their general medical history, demographics, undergo a physical examination as outlined in the eCRF, including:

- Height and weight determination and vital signs (pulse, respiration, blood pressure, temperature).
- Blood samples for testing (complete blood count [CBC], clinical chemistries, plasminogen activity and antigen, immunogenicity testing).
- Current medications
- All documented history of LC, including age of onset, diagnosis of Plasminogen 1 deficiency, duration of the disease, severity, past treatments (e.g., surgery, fresh or frozen plasma, laboratory grade plasminogen), responses to past treatments (e.g., regression, recurrence), and photographs of pseudomembranes if available in their medical history.
- A Screening ophthalmology examination will be completed. It will include photographs taken with a scale of both eyes, irrespective of pseudomembrane presence. A complete eye examination will be performed to determine the status of both eyes according to the Eye Examination form provided. Procedures for the Eye Examinations and the photographs are provided in an Ocular Manual, including tests for presence of corneal involvement. Subjects with corneal involvement will be noted in order to be considered separately in the efficacy assessment. These photographs will be taken with a scale and be provided to the independent assessors who will take measurements and determine the size of the pseudomembranes (when present). The independent assessors are ophthalmologists who review the photographs, data completed on the Eye Examination Form, measure the lesions (where applicable), [redacted]. All Eye Examination Forms and photographs are uploaded into a secure database with restricted access. The assessors do not review their own patient data.

All subjects will be screened for the presence of HAV Ab, HBsAg Ab, HBsAg, HCV Ab, HIV1-2 Ab, HIV1-2Ag(p24), and Parvovirus B19 Ab. In addition nucleic acid tests (NAT) will be performed for HBV, HCV and HIV 1-2. The NAT for HAV and Parvovirus B19 will be performed only in case of negative serology.

All HBV and/or HAV antibody negative subjects will be vaccinated against Hepatitis B and/or Hepatitis A prior to study entry. Subjects are not required to complete the second (i.e., booster) Hepatitis A vaccination, scheduled 6 months after the first injection, before study enrollment. Subjects who are too young (0 to 12 months old) to receive these vaccinations will not be excluded from study participation, but the risk, rationale, and plan for vaccination will be clearly explained in the informed consent/assent.

**Table 3: Screening Visit**

<table>
<tr><th colspan="4">Screening visit</th></tr>
<tr><th>Physical Assessment</th><th>Blood samples</th><th>Medical History, Medications</th><th>Ophthalmology</th></tr>
<tr><td>General PA per center standard of care<br>Height and weight<br>Demographics</td><td rowspan="2">Samples for:<br>• Hematology: Complete blood count [CBC]<br>• Clinical chemistries<br>• Plasminogen antigen and activity level<br>• Immunogenicity testing</td><td rowspan="3">• Duration of LC to date<br>• Severity of disease<br>• Age of onset<br>• Precipitating parameters<br>• Previous treatments for LC and responses<br>• Number and description of regressions to date<br>• Numbers of relapses to date<br>• Genetic testing results (if available)<br>• Current Medications</td><td>Complete eye examination, noting corneal involvement</td></tr>
<tr><td>Vital Signs:<br>• Pulse<br>• Respiratory rate<br>• Blood pressure<br>• Body Temperature</td><td>Measurement of pseudomembrane surface area, assessed by the independent ophthalmologists, if a pseudomembrane is present.</td></tr>
<tr><td></td><td>Viral screening via Serology:<br>• HAV Ab<br>• HBsAg Ab<br>• HBsAg<br>• HCV Ab<br>• HIV1-2Ag(p24)<br>• HIV1-2 Ab<br>• PV-B19 Ab<br>• Viral screening via NAT: HAV*<br>• HBV<br>• HCV<br>• HIV 1-2<br>• PV-B19*<br><br>Hepatitis A and B vaccination as needed</td><td>Photograph of pseudomembranes with scale on both eyes, even if pseudomembrane not present<br><br>Clinical assessment of pseudomembrane if present</td></tr>
</table>

** Only in case of negative serology*

All screening procedures will be performed within a 30-day window prior to receiving the first study IMP administration. Mailed Informed Consents ahead of the Screening Visit must show site processes and documentation for obtaining consent and allowing the subject to ask questions.

### 6.6.2 Subject Identification

All new subject enrollments will be immediately communicated to ARG.

Subjects who meet all the eligibility criteria, have signed and dated the informed consent(s)/assent(s) and have completed all screening activities will be assigned a unique identification number. The subject identification number will consist of a two digit study site number (e.g., 01) and a sequential 2 digit subject number (e.g., 05) reflecting the order in

which the enrollment takes place at that site. Thus, a subject coded as 01-05 will be the fifth subject enrolled in the study site Nr.1.

The study site is responsible to maintain a current log of subject number assignments in order to avoid assignment errors, such as duplication or skipping of numbers. The subjects' initials and unique identification number must be entered onto all study documents (i.e. eCRF, sample containers, drug accountability log, etc.).

**Baseline Visit (Enrollment)** The day of the first IMP administration (Day 0) will be considered the baseline visit. This visit is dependent upon the completion of a Screening visit within the preceding 30 days. All subjects will undergo a physical assessment and ophthalmologic examination, with measurement of pseudomembranes, where applicable. All enrollment criteria will be confirmed and the following parameters will be recorded prior to IMP administration:

- Physical Assessment including height and weight
- Pulse
- Respiratory rate
- Blood pressure
- Body temperature

Current medications will be reported in the subject's eCRF.

Any further history of LC, treatments, and response to treatments (including regression and recurrence) of each affected eye will be captured, since the Screening Visit.
A baseline ophthalmology examination which will be completed. It will include photographs taken with a scale of both eyes, irrespective of pseudomembrane presence. A complete eye examination will be performed to determine the status of both eyes according to the Eye Examination form provided. Procedures for the Eye Examinations and the photographs are provided in an Ocular Manual, including tests for presence of corneal involvement. Subjects with corneal involvement will be noted in order to be considered separately in the efficacy assessment. These photographs taken with a scale will be provided to the independent assessors who will take measurements and determine the size of the pseudomembranes (when present). The independent assessors are ophthalmologists who review the photographs, data completed on the Eye Examination Form, measure the lesions (where applicable), [redacted]. All Eye Examination Forms and photographs are uploaded into a secure database with restricted access. The assessors do not review their own patient data.

A pre-treatment serum sample from each subjects included in the clinical trial will be stored at a temperature below -70°C for later testing, if needed.

A blood sample will be withdrawn for genetic determination using plasminogen molecular analysis at screening (see 19.1 Appendix 1). This genetic test is voluntary, and subjects who do not consent to genetic testing will not be excluded from study participation. The molecular analysis of plasminogen will be performed to identify and study genetic mutations in the gene coding for plasminogen that may have caused Type 1 plasminogen deficiency.

Upon enrollment, subjects will be assigned to either Group **1** or Group **2** based on the presence of pseudomembranes. Subjects with at least one eye presenting

pseudomembranes will be assigned to Group **1**, while subjects with both eyes being asymptomatic will be assigned to Group **2**.

For dosage and treatment schemes please refer to

Table **2** and Section 8.5.

Subjects will be given detailed instructions regarding use of IMP during home administration. Study participants receiving the IMP will be provided with a Subject Manual with all the information about storing the IMP, returning used vials and/or unused vials as well as administration directions and a Subject Diary to collect data on home treatment. In addition they will capture an adverse events and concomitant medications in the Subject Diary. The subject's diary will be checked each time it is received at the site by the local study coordinators or PI to confirm the actual quantity of the IMP used, as well as to verify that the IMP is administered as directed against the vials returned to the site. Discrepancies between the IMP provided and that utilized must be discussed and reconciled. At completion of study or withdrawal of the participant, the subject's diary will remain at the site as part of the subject's source documentation. The Subject Diary will be completed and collected each 4 weeks at the clinic visit or if later in the study, mailed in. To encourage completion of the Subject Diary with adverse event, concomitant medication data reporting as well as IMP reporting, the Site Coordinator will call the subjects weekly to ensure subjects are completing their diaries and available to ask questions.

**Table 4: Baseline Visit**

| Baseline visit (Visit 0) | | | |
|---|---|---|---|
| **Physical Assessment** | **Blood samples** | **Medical History, IMP, AE and Medications** | **Ophthalmology** |
| General PA per center standard of care<br>Height and weight | Pre-treatment sample for later virology testing | Prior medication recording<br>Given a Subject diary. | Complete eye examination, noting corneal Involvement |
| Vital Signs:<br>• Pulse<br>• Respiratory rate<br>• Blood pressure<br>• Body Temperature | Blood sample for genetic determination (optional) | AE and concomitant medication recording immediately post administration | Measurement of pseudomembrane surface area, assessed by the independent ophthalmologists, if a pseudomembrane is present. |
| | | | Photograph of pseudomembranes with scale on both eyes, even if pseudomembrane not present<br>Clinical assessment of pseudomembrane if present |

### 6.6.3 Immunogenicity Testing

Immunogenicity studies will be carried out as part of the safety assessment. Blood samples will be obtained before starting IMP treatment at screening, at the end of Segment 1, every four weeks thereafter in Segment 2, every 2 months during continuation segment as well as the Termination Visits 1 and 2. The following markers will be evaluated:

1. Antibodies against human plasminogen,
2. Antibodies against bovine aprotinin.

Timing for required samples and evaluation parameters are specified in the table of events (Table ).

### 6.6.4 Interim Visits

Subjects will be required to visit the study site at least every 4 weeks for evaluation, up to the end of Segment 2, as determined by the protocol. If a subject undergoes surgery there will also be a Surgery Visit (Seg1SV) and another visit 2 weeks Post Surgery Visit to check for recurrence (Seg1PSV), described under section 6.6.6 and 6.6.7.

All subjects will undergo a physical assessment and ophthalmologic examination, with pseudomembrane photographs taken by the site and surface area measurements provided by the independent ophthalmologists. Procedures for the Eye Examinations and the photographs are provided in an Ocular Manual, including tests for presence of corneal involvement. Subject diaries will be reviewed by the study site.

Samples will be collected for immunogenicity, hematology and clinical chemistry testing and concomitant medications will be recorded. AEs will be reviewed by the study coordinator and/or PI in the subject diary as well as IMP administration and drug accountability reconciliation with the vials returned by the subject.

**Table 5: Interim Visits (Visits 1, 2)**

| Visits 1 (End of Segment 1) 2 (Week 4 of Segment 2) | | | |
|---|---|---|---|
| **Physical Assessment** | **Blood samples** | **IMP, AE and Medications** | **Ophthalmology** |
| General PA per center standard of care<br>Height and weight | Samples for:<br>• Hematology: Complete blood count [CBC]<br>• Clinical chemistries<br>• Immunogenicity studies | Given a Subject diary to be collected at each site visit depending on the Group and Segment.<br>Study site will call the subject weekly to follow up on status.<br>AE and concomitant data collection<br>Dispensing and reconciling IMP with what was dispensed, administered and returned. | Complete eye examination, noting corneal involvement |
| Vital Signs:<br>• Pulse<br>• Respiration<br>• Blood pressure<br>• Temperature | | | Measurement of pseudomembrane surface area, assessed by the independent ophthalmologists, if a pseudomembrane is present. |
| | | | Photograph of pseudomembranes with scale on both eyes, even if pseudomembrane not present<br>Clinical assessment of pseudomembrane if present |

### 6.6.5 Surgery Visit (Seg 1 SV)

Only Group 1 eyes that present partial or no pseudomembrane regression at the end of their first entry into Segment 1 will undergo surgery to remove the pseudomembranes, within 2 weeks. These eyes will be then be included in Group 1B for Study Segment 2 following surgery.

Prior to surgery following will be performed:

- A general Physical Assessment, including height and weight and vital signs will be performed.
- Concomitant Medications will be recorded as well as AEs.
- An ophthalmologic examination will be performed, with pseudomembrane photographs taken by the site and surface area measurements provided by the independent ophthalmologists. Procedures for the Eye Examinations and the photographs are provided in an Ocular Manual, including tests for presence of corneal involvement.
- The Subject Diary will be reviewed with the subject and a new diary issued with instructions on the new dosing regimen.
- Drug Accountability will be performed from the vials returned by the subject versus what has been dispensed to the subject.

Following the surgery (if possible), a further photograph will be taken to provide data for the independent ophthalmologists to use as the new baseline status of the pseudomembrane for their 2 week Post Surgery Visit.

Segment 2 will begin immediately after the surgery has taken place.

The dose will be amended as per the algorithm.

### 6.6.6 Post Surgery Visit (Seg 2 PSV)

Two weeks after surgery a clinic visit is conducted. At this visit the following will be performed:

- An ophthalmologic examination will be performed, with pseudomembrane photographs taken by the site and surface area measurements provided by the independent ophthalmologists. Procedures for the Eye Examinations and the photographs are provided in an Ocular Manual, including tests for presence of corneal involvement.
- Concomitant Medications will be recorded as well as AEs.
- The Subject Diary will be reviewed with the subject and a new diary issued with instructions on the new dosing regimen.
- Drug Accountability will be performed from the vials returned by the subject versus what has been dispensed to the subject.

*If the subject has pseudomembrane regression, the subject will be exited from the study.*

### 6.6.7 Continuation segment Follow-up visits

At the end of Study Segment 2, subjects will be given an option to continue treatment in the Continuation Segment, a long-term safety segment up to activation of Sponsor Initiated Expanded Access protocol or product availability.

Every 6 month from the beginning of Continuation segment, Follow Up clinic visits will be scheduled. At these visits all the assessments will be as per the interim visits described below and summarized in Table 5.

All subjects will undergo a physical assessment and ophthalmologic examination, with pseudomembrane photographs taken by the site and surface area measurements provided by the independent ophthalmologists. Procedures for the Eye Examinations and the photographs are provided in an Ocular Manual, including tests for presence of corneal involvement. At each clinic visit the Diary will be reviewed and collected by the investigator and a new Diary provided to the subjects. Phone calls will be performed monthly after each clinic visit to remind the subjects to complete the Diary.

The Diary is the source for collection of these data; therefore, it is critical that the subject or subject's parent(s)/guardian(s) completes the Diary correctly. The subject or subject's parent(s)/guardian(s) should be trained on how and when to complete each field of the Diary.

Subject Diary training should be directed at the individual(s) who will report adverse events and who will enter the information into the Diary. This individual may not be the subject or subject's parent(s)/guardian(s), but if a person other than the subject or subject's parent(s)/guardian(s) enters information into the Diary, this person's identity must be documented in the Third Party Delegation section of the Subject Diary. Any individual that writes in the Subject Diary must receive training on completion of the Diary Card at the time of the visit. This training must be documented in the subject's source record.

During the phone call, specific questions on safety will be addressed to document any possible adverse event observed.

Samples will be collected for hematology and clinical chemistry testing and concomitant medications will be recorded.

AEs will be reviewed by the study coordinator and/or PI in the subject diary as well as IMP administration and drug accountability reconciliation with the vials returned by the subject.

During the Continuation Segment, blood samples will be taken every 2 months for immunogenicity evaluation.

During Continuation Segment, a Home Health Agency may be utilized to collect the samples outside of the investigational site. The use of the Home Health Agency is optional. Subjects that opt to use the Home Health Agency must consent prior to initiation of the use of the Home Health Agency for that subject.

At each time point and starting from the approval of Amendment 4 of this clinical protocol, an extra blood sample must be obtained and stored for possible additional immunogenicity investigation.

### 6.6.8 Unscheduled Site Visits

During Segments 2 in Groups 1A, 1B or 2 if at any time the subject reports a regression or recurrence of a pseudomembrane an unscheduled site visit will be required for confirmation by the site of the presence of a pseudomembrane. At this visit the following will be performed:

- An ophthalmologic examination will be performed, with pseudomembrane photographs taken by the site and surface area measurements provided by the independent ophthalmologists. Procedures for the Eye Examinations and the photographs are provided in an Ocular Manual, including tests for presence of corneal involvement.
- Concomitant Medications will be recorded as well as AEs.
- The Subject Diary will be reviewed with the subject and a new diary issued with instructions on the new dosing regimen, (if applicable).
- Drug Accountability will be performed from the vials returned by the subject versus what has been dispensed to the subject.

In addition to the Clinic Visits to be performed every 6 month, from the beginning of continuation segment, the patients' clinical management during this segment will be done at the investigator's medical practice. Unscheduled visits can be conducted and the continuation subjects may be required to come to the site for an unscheduled visit at any time for safety reasons, and/or recurrence of pseudomembranes. This will be at the discretion of the Investigator and all, or some of the selected tests above could be completed, based on the Investigator's assessment.

*If the subject has pseudomembrane recurrence, anytime after 2 weeks of the Start of Segment 2 in Group 1A, 1B or 2, then the subject will be re-entered into Group 1 Segment 1*

*for Group 1A and Group 2. If in Group 1B the subject re-entered into the 1B dosing regimen but a second surgery will not be performed.*

### 6.6.9 Visit 3 Study Termination #1 [T1] .

Visit 3 is a termination visit (Termination #1 [T1]) to mark the end of efficacy evaluations at the end of Segment 2. At Visit 3, all subjects will undergo:

- A general Physical Assessment, including height and weight and vital signs will be performed.
- Concomitant Medications will be recorded as well as AEs.
- An ophthalmologic examination will be performed, with pseudomembrane photographs taken by the site and surface area measurements provided by the independent ophthalmologists. Procedures for the Eye Examinations and the photographs are provided in an Ocular Manual, including tests for presence of corneal involvement.
- The Subject Diary will be reviewed with the subject and a new diary issued with instructions on the new dosing regimen.
- Drug Accountability will be performed from the vials returned by the subject versus what has been dispensed to the subject.
- Samples will be collected for immunogenicity, hematology and clinical chemistry testing and concomitant medications will be recorded.
- Samples will also be taken for future virology testing, if needed.

**Table 6: Visit 3/Termination #1 [T1] Schedule**

| Visit 3/Termination #1 (T1) | | | |
|---|---|---|---|
| **Physical Assessment** | **Blood samples** | **IMP, AE and Medications** | **Ophthalmology** |
| General PA per center standard of care<br>Height and weight | Samples for:<br>• Hematology: Complete blood count [CBC]<br>• Clinical chemistries<br>• Immunogenicity studies | Collect the Subject Diary and issue a new one which will be mailed in every 4 weeks throughout the continuation phase.<br>Study site will call the subject weekly to follow up on status.<br>AE data collection and Concomitant data collection via the monthly Subject Diaries mailed to the site.<br>Dispensing and reconciling IMP with what was dispensed, administered and returned. This will be achieved through mailing the IMP to the site per the Subject Manual instructions.<br>If subjects exit the study at this visit the IMP accountability will be the final reconciliation. | Complete eye examination, noting corneal involvement |
| Vital Signs:<br>• Pulse<br>• Respiratory rate<br>• Blood pressure<br>• Body Temperature | Viral screening via Serology<br>• HAV Ab<br>• HBsAg Ab<br>• HBsAg<br>• HCV Ab<br>• HIV1-2Ag(p24)<br>• HIV1-2 Ab<br>• PV-B19 Ab<br>Viral screening via NAT:<br>• HAV*<br>• HBV<br>• HCV<br>• HIV1-2<br>• PV-B19* | | Measurement of pseudomembrane surface area assessed by the independent ophthalmologists, if a pseudomembrane is present.<br>Photograph of pseudomembranes with scale on both eyes, even if pseudomembrane not present<br>Clinical assessment of pseudomembrane if present |

** Only in case of negative serology*

### 6.6.10 Visit 4 Follow-up visit

All subjects exiting the study during Segment 1 and 2 or afterwards, regardless of the reason for exit, will be asked to consent in writing to be followed for 6 months after study exit. If they consent, they will be given a Subject Diary to record any change in disease course, including relapses, spontaneous regressions, surgeries, medications, and other changes in clinical status. During the 6 months follow up period subjects will not be treated with the IMP. At the end of 6 months, each subject will return to the study site, submit the diary to study staff, and undergo complete clinical assessments on disease status and safety with a Physical Assessment,(Table 7). This visit will be Visit #4.

The subjects will mail their subject diaries to the site and the site will send subject diaries for each month and call the subjects weekly to ensure the subjects are completing the diaries.

**Table 7: Visit 4/Follow-up Visit Schedule (for subjects who exit after Segment 1 or 2)**

| Visit 4/Follow-up Visit | | | |
|---|---|---|---|
| **Physical Assessment** | **Blood samples** | **IMP, AE and Medications** | **Ophthalmology** |
| General PA per center standard of care<br>Height and weight | | Collection of the Final Subject Diary<br>final reconciliation of IMP with what was dispensed, administered and returned throughout the study duration. | Complete eye examination, noting corneal involvement |
| Vital Signs:<br>• Pulse<br>• Respiratory rate<br>• Blood pressure<br>• Body Temperature | | | Measurement of pseudomembrane surface area assessed by the independent ophthalmologists, if a pseudomembrane is present. |
| | | | Photograph of pseudomembranes with scale<br>on both eyes, even if pseudomembrane not present<br>Clinical assessment of pseudomembrane if present |

### 6.6.11 Reminder calls

Reminder calls will be performed monthly, between each 6-month safety .

### 6.6.12 Termination Visit #2 [T2]

At the Continuation Segment termination visit (Termination #2 [T2]), all subjects will undergo an immunogenicity assessment and ligneous pseudomembranes presence evaluation. A limited physical assessment and ophthalmologic examination, with pseudomembrane presence evaluation will be performed and blood samples will be obtained for CBC and clinical chemistries tests. The Subject Diary will be collected and reviewed by the investigator

Blood samples will be taken for immunogenicityevaluation.

The subjects will also return to the site the used IMP vials with the associated forms for drug accountability purposes. Samples will also be taken for the following tests in viral safety.

- HAV Ab
- HBsAg Ab
- HBsAg
- HCV Ab
- HIV1-2Ag(p24)
- HIV1-2 Ab
- PV-B19 Ab
- Viral screening via NAT: HAV*
- HBV
- HCV
- HIV1-2
- PV-B19*

At both termination visits, a termination case report form will be completed. The primary reason for termination will be listed on the eCRF. One of the following conditions should be met:

- Subject completed the protocol.
- Subject experienced adverse drug reaction(s) from the study medication necessitating discontinuation of treatment.
- Subject was withdrawn by the Investigator at the site for non-IMP related reasons.
- The study monitor assigned to the site or the clinical manager for the study should be notified before any subject is withdrawn.
- Unsatisfactory therapeutic response to study product, e.g. pseudomembrane progression, development of corneal involvement, as determined by the investigator. (Subjects who exit the trial as study failures would fulfil this criterion).
- Subject or legally authorized representative voluntarily withdrew consent.
- Subject died.
- Other.

**Table 8: Termination Visit #2 [T2] Schedule**

| Termination Visit #2 Schedule (T2) | | | |
|---|---|---|---|
| **Physical Assessment** | **Blood samples** | **IMP, AE and Medications** | **Ophthalmology** |
| General PA per center standard of care<br>Height and weight | Samples for:<br>• Hematology - CBC<br>• Clinical chemistries<br>• Immunogenicity studies | Prior and Concomitant medication recording<br>Collection of final Subject diary<br>Concomitant medication and AE data collection<br>Final Reconciliation of IMP with what was dispensed, administered and returned including the last unused vials from the subject's home. | Complete eye examination, noting corneal involvement |
| Vital Signs:<br>• Pulse<br>• Respiratory rate<br>• Blood pressure<br>• Body Temperature | Viral screening via Serology<br>• HAV Ab<br>• HBsAg Ab<br>• HBsAg<br>• HCV<br>• HIV1-2Ag(p24)<br>• HIV1-2 Ab<br>• PV-B19 Ab<br>Viral screening via NAT:<br>• HAV*<br>• HBV<br>• HCV<br>• HIV1-2<br>• PV-B19* | | Measurement of pseudomembrane surface area assessed by the independent ophthalmologists, if a pseudomembrane is present.<br>Photograph of pseudomembranes with scale on both eyes, even if pseudomembrane not present<br>Clinical assessment of pseudomembrane if present |

** Only in case of negative serology*

Regardless of the reason for study termination, all data available for the subject up to the time of termination will be recorded onto the appropriate eCRF, and all subjects' diary cards will be collected for auditing by the study monitor, prior to retrieval by Kedrion S.p.A.

### 6.6.13 Early Withdrawal from the Study

See Section 6.4.3 - Removal of Subjects from Treatment or Assessment including but not limited to:

- Withdrawal of Consent
- Safety concerns
- Subject non-compliance
- Lack of efficacy

Subjects will be required to return all unused product to the study center.

### 6.6.14 End of study

For the purpose of this protocol, end of study is defined as the last safety follow-up information (immunogenicity results) received by investigators.

## Table 9: 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 Continuation 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 Continuation 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

### 6.6.15 Clinical Laboratory Measurement

Blood will be obtained from all subjects for laboratory testing (hematology, clinical chemistries) at the following time points:

- Screening (if the screening visit falls outside of the 30 day window period, screening laboratory evaluations will be repeated and documented to be within eligibility requirements prior to entry)
- Initial 4 weeks evaluation in Segment 1
- Every 4 weeks during the subsequent weeks study period in Segment 2.
- Study termination (T1 and T2)

Hematology and clinical chemistry tests will be performed on Ethylene diaminetetra acetic Acid (EDTA) anti-coagulated whole blood and serum, respectively, in the laboratories specified by each PI. For Europe it will be performed by a local laboratory specified by the PI, in the US a Central Laboratory will be utilized. The tests will include a CBC [consisting of hemoglobin (Hgb), hematocrit (Hct), red blood cell count (RBC), white blood count (WBC), platelet count] and a clinical chemistry panel [consisting of sodium, potassium, chloride, total protein, albumin, alanine aminotransferase (ALT), total and fractionated bilirubin, alkaline phosphatase, blood urea nitrogen (BUN), creatinine and glucose].

At screening, serologic tests for anti-HAV, anti-Parvovirus B19, anti-HCV, anti-HIV1-2 and anti-HBsAg antibodies, HBsAg, and HIV1-2Ag(p24) will be performed. For parvovirus B19 and HAV, in case of negative results, additional NAT testing will be performed. All HBV and/or HAV test-negative subjects, except children under 1 year of age, will be vaccinated against Hepatitis B and/or Hepatitis A. All serologic tests will be repeated at the end of the study for all subjects that received the IMP (at least once).

Immunogenicity testing for antibodies against human plasminogen and bovine aprotinin will be performed according an assay method at the following time points:

- Screening
- Initial 4 weeks evaluation in Segment 1
- Every 4 weeks thereafter in Segment 2
- Every 2 months during Continuation Segment
- Study termination

At each time point and starting from the approval of Amendment 4 of this clinical protocol, an extra blood sample must be obtained and stored for possible additional immunogenicity investigation.

Plasminogen antigen and activity will be tested at the following time points:

- Screening

Immunogenicity testing and plasminogen testing will be performed at a specified central laboratory (see Appendix 2).

- Immunogenicity testing, using Enzyme-Linked Immunosorbant Assays (ELISA).
- Plasminogen Activity using a chromogenic assay
- Plasminogen antigen using a nephelometric immunoassay

### 6.6.16 Laboratory Range.

The normal range for Immunogenicity (PLG and Aprotinin) are 0.082 – 0.142 OD and ≤15 U/mL, respectively. For the diagnosis of Type 1 Plasminogen deficiency, the following range for plasminogen antigen and activity are taken: < 0,09 g/L and < 51%, respectively. For the general safety laboratory assessments the normal laboratory ranges are provided by each center and copies will be stored in the Trial Master File.

### 6.7 Quality Assurance

The present clinical trial sponsored by Kedrion S.p.A. will be conducted in accordance with the ICH GCP guidelines. The clinical and regulatory teams will systematically control the essential documents generated during this trial.

All segments of the trial will be monitored by the clinical team with the critical segments of this trial, particularly the starting and the ending of the trial, being subject to internal audits by Quality Assurance (QA). The Investigator will allow the auditors access to subjects' source data and to study documentation, and study site staff must be available for discussion. All clinical study monitoring visits and inspections by QA will be followed by internal reports and corrective actions, if needed. Follow-up letters will be forwarded to sites after all clinical visits.

Regulatory Authorities, such as European CA or the US FDA, may also perform study audits. The Investigator will allow the CA inspectors access to Subjects source data and to study documentation. The PI and clinical site staff will be available for discussion.

# 7 CRITERIA FOR SAFETY EVALUATION

**Vital signs**
Safety will be assessed by recording vital signs (blood pressure, body temperature, pulse and respiratory rate), serology and blood chemistries according to the schedule reported in the previous section.

**Immunogenicity**
Immunogenicity studies will be carried out as part of the safety assessment. Samples will be taken before starting the treatment during screening for all subjects, then for Group 1 and Group 2 after the 4 week evaluation in Segment 1, and every 4 weeks thereafter in Segment 2, as well as every 2 months during continuation segment and Termination Visits 1 and 2. The following markers will be measured: 1) antibodies against human plasminogen, 2) antibodies against bovine aprotinin.

**Viral safety**
A pre-treatment serum sample will be obtained at the baseline and stored at -70ºC or below for possible future testing. At screening for all patients, serologic tests for anti-HAV, anti-Parvovirus B19, anti-HCV anti-HIV1-2 antiHBsAg antibodies, HBsAg and HIV1-2Ag(p24) will be performed in addition to NAT testing and repeated at the end of Segment 2 (V3/T1) and (V6/T2) for all subjects enrolled.
The number of subjects with conversion from negative to positive during the study will be reported in the study results.

**Adverse events**

Evaluation of safety includes the analysis of adverse events. The severity, seriousness, and relatedness of AEs to the study drug will be observed on repeated administrations of the study drug. All AEs will be listed and summarized by body system and treatment group. SAEs will be similarly summarized. The number and proportion of subjects who have one or more AEs, and who have one or more temporally related AEs, will be presented.

## 7.1 Adverse Events (AEs)

### 7.1.1 Adverse Event (AE)/Adverse Drug Reaction (ADR)

An **adverse event** is "any untoward medical occurrence in a Subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment."

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product;
- Any new disease or exacerbation of an existing disease;
- Recurrence of an intermittent medical condition (e.g., headache) not present at baseline;
- Abnormal laboratory values or other clinical tests (e.g. electrocardiogram or x-ray) that result in symptoms, a change in treatment, discontinuation from study drug, or are considered to be medically significant.

Any subject reporting an adverse event will be examined by a physician as soon as possible. The physician in attendance will do whatever is medically necessary for the safety and well-being of the subject. The subject will remain under observation as long as clinically indicated, in the opinion of the investigator. All abnormalities will be followed until resolved or until medically stabilized.

### 7.1.2 Assessment and Documentation

- AEs whether related or not, will be collected via spontaneous reporting by the subject at any time beginning from the time of first dose of IMP until **30** days post dose. AEs will be collected to include all the months included in the continuation segment, up to activation of Sponsor Initiated Expanded Access protocol.
- A surgery to remove pseudomembranes is not considered an AE for this study.

At every visit, the occurrence of AEs will be assessed by study staff. AEs can be identified from safety evaluations conducted throughout the trial and by the following methods:

- reviewing subject case histories;
- reviewing any clinically significant changes in the findings of physical examinations, vital signs or objective laboratory tests;
- signs observed by the study staff should also be considered to elicit AEs, especially when the subject cannot be asked (e.g. the subject is anesthetized);

Certain SAEs may be related to the drug administration. The recommended drug administration must be closely followed. Subjects must be carefully monitored and observed for any symptoms throughout the study. Rarely, in isolated cases, severe reactions as shock

or anaphylaxis may occur; in these cases the IMP administration should be stopped immediately and medical attention should be sought.

AEs assessed as non-serious will be reported through the 30 days following the subject's last study drug dose. Non-serious AEs must be followed until resolution, or for 30 days after the subject's last study drug dose, whichever comes first.

### 7.1.3 Severity of Adverse Events

The Investigator will assess the severity of AEs according to the criteria below:

- **Mild**: The AE is transient and does not interfere significantly with the subject's normal functioning level. The AE resolves spontaneously or may require minimal therapeutic intervention.
- **Moderate**: The AE produces limited functional impairment and may require therapeutic intervention. The AE produces no sequelae.
- **Severe**: The AE results in significant impairment of function and may lead to temporary inability to resume the subject's normal life pattern. The AE produces sequelae which requires prolonged therapeutic intervention.

### 7.1.4 Causality Assessment

The relationship of treatment to adverse event will be rated by the Investigator using the scale below:

- **Definitely related**: A clinical event (including laboratory test abnormality) occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (de-challenge) should be clinically plausible. The event must be definitively associated pharmacologically or phenomenologically, using a satisfactory re-challenge procedure, if necessary.
- **Probably related**: A clinical event (including laboratory test abnormality) with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Possibly related**: A clinical event (including laboratory test abnormality) with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
- **Not related**: An event for which sufficient information exists to conclude that the etiology of the event is unrelated to the IMP.

The Investigators will assign causality at their respective sites during the study. The Sponsor's Medical Representative for the study will review the assigned causality for all AEs and SAEs prior to database lock. This assignment will be included in the study database and final study report.

### 7.1.5 Serious Adverse Events (SAEs)/Adverse Drug Reactions (SADRs)

All AEs must be evaluated as potential **serious adverse events**. A SAE or a SADR is "any untoward medical occurrence that at any dose:

- Results in death;
- Is life-threatening;
  - The subject was at immediate risk of death from the AE as it occurred.
  - This does not include an event that, has it occurred in a more severe form or was allowed to continue, might have caused death.
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapability;
- Is a congenital anomaly/ birth defect (occurring in the child of a subject who was exposed to the IMP); or
- Is a medically important event or reaction.
  - Events that did not result in death or hospitalization but may, based on appropriate medical judgement, jeopardize the subject or require intervention to prevent one of the outcomes listed above, should also be considered SAEs.

All AEs assessed as serious must be reported beginning from the time of first dose of IMP until 30 days post dose. SAEs must be followed until the event resolves, the event or sequelae stabilize, or it is unlikely that additional information can be obtained after demonstration of due diligence with follow-up efforts (i.e., the subject or Investigator is unable to provide additional information or, the subject is lost to follow-up).

A **non-serious** adverse event or adverse drug reaction is any AE/ADR that does not meet the above stated criteria.

### 7.1.6 Unexpected Adverse Reactions

An unexpected adverse reaction is "an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product)."

## 7.2 Reporting Rules

The investigator will evaluate all adverse events as to their severity and relationship to the IMP, and will report outcome and action taken. Where a diagnosis is possible, it is preferable to report this rather than a series of terms relating to the diagnosis. When reporting a syndrome, indicate all the associated signs and symptoms following the syndrome rather than each separate event. Sponsor/ARG will perform the MedDRA coding.

All AEs (serious and non-serious) will be recorded on the subject's eCRF. A cluster of signs and symptoms that result from a single cause should be reported as a single AE (e.g., fever, elevated white blood cell count, cough, abnormal chest x-ray, etc. can all be reported as "pneumonia").

### 7.2.1 Serious Adverse Event/Serious Adverse Drug Reaction Reporting

All SAEs must be reported to the ARG Pharmacovigilance Department (PVD) within **24 hours** of the Investigator becoming aware of the event. Sites may contact PVD by telephone, fax or email using the SAE Report Form.

**24-hour Safety Reporting Numbers**

**Toll Free Phone** [redacted]

**Toll Free Fax:** [redacted]

It should be noted that reporting to ARG PVD within 24 hours of awareness is required for all SAEs regardless of their causal relationship with a medicinal product.

If the SAE Report Form cannot be completed within 24 hours of awareness, the Investigator or designee must telephone or email ARG PVD to report the following minimum information:

1. Name, title, and contact information of person reporting event;
2. Subject number;
3. Protocol number;
4. Name of IMP;
5. Date and time of most recent administration of Investigational Product;
6. Date and time of start of SAE;
7. Description of SAE (symptoms or disease/syndrome);
8. Severity of SAE;
9. Assessment of Causality.

In all cases, the Investigator or designee must submit a completed SAE Report Form to ARG PVD within 24 hours of notifying ARG PVD of the event by telephone or e-mail.

The ARG PVD, together with the Sponsor's Medical Representative, will review all safety information/ documentation and follow up with the investigative site to obtain any other required information. Follow-up information should be actively sought by the Investigator and reported to ARG PVD as it becomes available using the SAE Report Form.

The Sponsor and ARG are responsible for reporting the SAE to the authorities (e.g. the FDA in the US).

### 7.2.2 Additional Reporting Requirements for the Development of anti-plasmingoen and anti-aprotinin antibodies

Any development or increase in the titer (≥20%) of anti-aprotinin antibodies or anti-human plasminogen antibodies should be reported as an AE.
The principal investigators will be instructed to report all occasions of positive, anti-human plasminogen antibodies to Kedrion no later than 48 hours from the time of awareness. All the positive anti-plasminogen tests will be treated as expedited even if clinical evidence indicates that this finding does not represent a neutralizing antibody

### 7.2.3 Additional Reporting Requirements for Pregnancies

Pregnancy occurring during a clinical investigation, although not considered a serious adverse event, must be reported to the ARG Pharmacovigilance Department within the same timelines as SAEs. All female subjects of childbearing potential will be instructed to inform the Investigator immediately if she becomes pregnant during the trial. Both mother and fetus should be monitored carefully throughout the pregnancy to birth of the neonate, if possible, to obtain the maternal and fetal outcome and evaluate if there are any risks of AEs/SAEs associated with the IMP due after in utero exposure. All initial and follow-up reports of pregnancy should be reported using the Pregnancy Report Form. If no AEs are identified during the pregnancy or at the time of birth, the case will be closed. Any AE and/or abnormal pregnancy outcome identified in the mother, the fetus and/or the newborn, at any stage of pregnancy, meeting seriousness criteria should be reported as an SAE using the SAE Report Form. The case will be investigated as any other SAE and a neonate SAE case will be created and linked to the mother's file.

The following pregnancy/fetal outcomes are considered an SAE, which requires expedited reporting to the ARG Pharmacovigilance Department and should be reported immediately, no later than 24 hours from receipt of the information:

- Reports of congenital anomaly(ies) in the fetus, child
- Reports of late fetal death
- Reports of spontaneous abortion
- Reports of ADRs in a newborn/neonate that are fatal, life-threatening, resulting in persistent or significant disability/incapacity or resulting in or prolonged hospitalization.

### 7.2.4 Expedited Suspected Unexpected Serious Adverse Event (SUSAR) Reporting (Sponsor Responsibility)

The Sponsor will submit a written Investigational New Drug (IND) Safety Report (i.e., completed FDA Form 3500 A) to the responsible new drug review division of the FDA for any observed or volunteered adverse event that is determined to be:

1. Associated with the investigational drug or study treatment(s);
2. Serious; and
3. Unexpected.

Each IND Safety Report will be prominently labeled, "IND Safety Report," and a copy will be provided to all participating site Investigators.
Written IND Safety Reports will be submitted to the FDA as soon as possible and, in no event, later than 15 calendar days following the Sponsor's/ARG receipt of the respective adverse event information.

For each written IND Safety Report, the Sponsor will identify all previously submitted IND Safety Reports that addressed a similar adverse event experience, and will provide an analysis of the significance of newly reported adverse event in light of the previous, similar report(s).

If the results of the Sponsor's follow-up investigation show that an adverse event that was initially determined to not require a written IND Safety Report does, in fact, meet the requirements for reporting; the Sponsor will submit a written IND Safety Report as soon as possible, but in no event later than 15 calendar days, after the determination was made.

Follow-up information to an IND Safety Report will be submitted to the applicable review division of the FDA as soon as the relevant information is available.

In addition to the subsequent submission of a written IND Safety Report (i.e., completed FDA Form 3500A), the Sponsor will notify the responsible review division of the FDA by telephone or facsimile transmission of any observed or volunteered adverse event that is:

1. Associated with the use of the investigational drug or study treatment(s);
2. Fatal or life-threatening; and
3. Unexpected.

The telephone or facsimile transmission of applicable IND Safety Reports will be made as soon as possible but in no event later than 7 calendar days after the Sponsor's/ARG' initial receipt of the respective human adverse event information.

### 7.2.5 Reporting to Investigational Review Board/Ethics Committee

The Investigator will report to the IRB/EC in accordance with the respective IRB/EC policies and procedures and will submit any observed or volunteered adverse event that is determined to be:

1. Associated with the investigational drug or study treatment(s);
2. Serious; and
3. Unexpected to the IRB/EC in accordance with the respective IRB/EC policies and procedures.

AEs should be reported to the IRB as soon as possible, but no later than 10 calendar days following the Investigator receipt of the respective information. However, AEs meeting the following criteria must be reported to the IRB/EC within 24 hours of the Investigator receipt of the respective information:

1. Associated with the investigational drug or study treatment(s);
2. Fatal or life-threatening; and
3. Unexpected.

If the results of an Investigator's follow-up investigation show that an adverse event that was initially determined to not require reporting to the IRB/EC does, in fact, meet the requirements for reporting, the Investigator will report the adverse event to the IRB/EC as soon as possible, within the timeframes outlined above.

Follow-up information to a reported adverse event will be submitted to the IRB/EC as soon as the relevant information is available.

## 7.3 Laboratory Abnormalities

The investigator must assess the clinical significance of all abnormal laboratory values as defined by the compendium of normal values for the reference laboratory. Any clinically significant abnormalities should be fully investigated. "Clinically significant" is defined as any abnormality that the investigator feels is of major clinical concern, requires medical intervention, or that otherwise meets the definition of a "serious" AEs. Additional tests and other evaluations required to establish the significance, or etiology of an abnormal result or to monitor the course of an adverse event should be obtained when clinically indicated. Any abnormal value that persists should be followed at the discretion of the investigator. Clinically significant abnormal findings will be documented on the appropriate eCRF.

### 7.4 Concurrent Illnesses and Pre-existing Diseases

Intercurrent illnesses (including signs/symptoms of a pre-existing disease state) that are present at or before IMP administration, and that manifest with the same severity, frequency or duration subsequent to IMP administration, must be recorded on the relevant form in the eCRF. However, cases where there is an increase in severity or duration of the concurrent illness or pre-existing disease must be reported as AEs on the appropriate section of the eCRF.

Serious cases of increase in the subject's pre-existing conditions, including pseudomembrane progression, must be carefully monitored, recorded and discussed in the relevant study reports, but are not required to be reported in an expedited manner, unless the investigator considers them to be a major clinical concern.

# 8 CRITERIA FOR EVALUATION

### 8.1 Efficacy Evaluation

Based on published cases of LC, pseudomembranes always recur after surgical excision if left untreated. The primary efficacy endpoint in this study will be measured by the number of eyes that develop recurrent ligneous membranes in Segment 2 after initially showing total regression after treatment or after surgical excision.

Treatment with the IMP will be considered effective if the rate of pseudomembrane recurrence is numerically lower than the rate of recurrences in the subjects' historical data, with each evaluated eye compared with its own history of recurrence specific for left or right eye, dependent on the data available. If not available the standard expectation of the independent assessors will be used. Efficacy outcomes will be classified into 3 categories: complete success (no recurrence by the end of Segment 2), partial success (recurrence observed 2 weeks or more after 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 (recurrence within 2 weeks of start of Segment 2 or if at repeat cycles of Segment 1 for Group 1A, the pseudomembranes do not regress after Segment 1).

No formal statistical analysis for efficacy can be conducted because of the rarity of the disease and the small sample size. Additionally, a formal value for efficacy criterion cannot be set due to a lack of published literature describing the efficacy of any LC treatments. Therefore it is not possible to define the difference in the recurrence rate between historical and treatment period.

Secondary efficacy will be measured by the size of pseudomembrane regression.

1. Percentage decrease in the objective measurement of pseudomembrane surface area from baseline to the end of Segment 1.

   The objective measurement will be performed by taking scaled photographs of the eyes and comparing the pictures taken at the end of the each at the study visit as detailed in the study protocol. The photographs will be evaluated by at least 2 ophthalmologists

[redacted]

2. Proportion of eyes with a reduction of the overall membranes surface area as measured with the method above at the end of Segment 1.
3. Time to pseudomembrane recurrence after complete regression or surgery will be measured in days.

The evaluable populations for efficacy and safety analyses are defined in Section 8.1.

### 8.2 Safety Assessment

1. Percentage of subjects who develop antibodies against human plasminogen.
2. Percentage of subjects who develop antibodies against bovine aprotinin.
3. Percentage of subjects who experience signs and symptoms of sensitization.
4. Percentage of subjects who experience AEs (correlation with the IMP assessed as possible, probable, or definite).

Safety analysis population will include all subjects who receive at least one dose of IMP.

### 8.3 Follow Up Period Interim Analysis Assessment

An interim analysis will be performed and included into a Clinical Study Report which will be issued at the end of Segment 2 for all subjects enrolled in the study. It will be submitted to the FDA as part of a BLA submission.
A final analysis will be performed including data collected during the Continuation Segment (treated with IMP) and during the follow up period from exited subjects (not treated with IMP, but followed for any change in disease course, including relapses, spontaneous regressions, surgeries, medications, and other changes in clinical status). The final clinical study report will be provided at the end of the study.

# 9 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

### 9.1 Analysis Populations

***Intent to Treat (ITT) population***

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

***Modified ITT (mITT) population***

The 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 (PP) population***

The PP 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.

***Safety Population***

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

Efficacy analyses will be based on mITT and PP population.

## 9.2 Data Analysis

All the baseline, safety and efficacy endpoints will be summarized using descriptive statistics (mean, standard deviation, median, 25$^{th}$ percentile and 75$^{th}$ percentile [for quantitative parameters] and proportion [for qualitative variables]). Historical data on subjects' past LC treatment before enrolling in this study, including treatment received, pseudomembrane regression, recurrence, and time to recurrence (specific for left or right eye, dependent on the data available), will be presented. Further details will be described in the Statistical Analysis Plan (SAP).

## 9.3 Determination of Sample Size

The sample size of at least 10 subjects, for approximately of 20 evaluable eyes, has been selected by the treating investigators as a reasonable size to investigate since the disease prevalence is so low.

## 9.4 Efficacy

Efficacy responses will be summarized and presented on the basis of each 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. Confidence intervals for the recurrence rate (complete success, and complete plus partial success) will be calculated on the assumption o

Subjects' history of LC treatment and pseudomembrane regression and recurrence will be presented along with study data descriptively. No statistical comparisons will be conducted.

The secondary endpoint (regression rate) will also be presented as a frequency table, showing the numbers of eyes showing 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 intent to treat and per protocol populations (if different).

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.

### 9.5 Safety

The assessment of safety will be based on individual study subjects, except for local tolerability assessments which 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 the latest version of MedDRA®. 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.

### 9.6 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 end of Segment 1, every 4 weeks during Segment 2 as well as every 2 months during continuation segment and the 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.

# 10 PRODUCT ACCOUNTABILITY

The IMP administrator must maintain accurate records of dates and quantities, and lots of product received, to whom dispensed (subject-by-subject accounting), and accounts of any product accidentally or deliberately destroyed. The Investigator must retain all unused or expired product until the study monitor has confirmed accountability data.

An IMP Manual will be supplied to the site with detailed instructions including all logs and instructions on administration and how to handle the used and unused vials to enable complete and accurate drug accountability. A Subject Manual will be supplied to the subject's with instructions on receipt, storage and accountability of IMP.

The subject shall be given a subject's diary to collect data on administrations occurring at home. The subject's diary will be checked during the study to confirm the actual quantity of the IMP used, as well as the frequency of administrations. Subjects will be given strict instructions about use of IMP in case of home administration. Any discrepancies between the IMP provided and actually used must be discussed sand justified. The subject's diary will remain at the site as part of the subject's source record.

At the conclusion of IMP administration, all unused IMP supplies will be returned to Kedrion S.p.A. An overall summary of all IMP supplies received, used, wasted, and returned must be prepared at the conclusion of the study.

## 10.1 Management of Investigational Product

The IMP must be administered according to the procedures described in the present protocol. Only subjects enrolled in the study are allowed to receive IMP administrations. The IMP must be stored at a temperature of [redacted]

## 10.2 Updates to Investigators

Kedrion S.p.A. will immediately inform the investigators of any new information about the IMP that may have an impact on study conduct or on subject safety.

# 11 EVALUATION OF LABORATORY RESULTS

All laboratory results will be evaluated by an expert physician who may require the repetition of the test. Any decision taken on the basis of the obtained results must be documented in the eCRF and in the subject's source data. In case the results met the definition of AE/SAE they must be managed according to the procedures stated in the protocol. The PI must assign "Clinically Significant" and/or "Non Clinically Significant" determinations to out of range laboratory results.

# 12 CASE REPORT FORM (CRF)

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.

Also the subjects (or their legal representatives) should allow access to their medical records. This request for access will be explained when the subjects supply their signed informed consent. Authorization must be received for participation in the study.

The following data will be reported directly on the eCRF and will be considered as original data: demographic data, subject's history, simultaneous intake of drugs, AEs and study conclusion.

# 13 STUDY MONITORING AND COMPLIANCE

Kedrion S.p.A. will designate a study monitor to oversee study compliance to the applicable guidelines and regulations. The monitor will ensure that data collected are faithful, accurate and complete, that the rights and safety of the subjects are assured and that there are minimal deviations from the protocol.

The monitor will inspect the study sites on a regular basis and according to pre-established intervals. The purpose and frequency of the visits may be modified in the course of the study in connection with the conduction or any problems occurring.

The Investigator must be available to clinical study monitors during their visits and must ensure that the clinical study monitor has access to all documents that they require, including the subject's files (direct access).

The Investigator agrees to cooperate with the clinical study monitor to make certain that any problems detected in the course of these monitoring visits are resolved.

The anonymity of the subject must be safeguarded and all data checked during these monitoring visits must remain confidential.

## 13.1 Visit Window

Due to the numerous visits required in this study and the difficulties with scheduling of subjects traveling distances to get to the sites, visit window guidelines have been established.

Baseline Visit ± 3 days

Visit 1 ± 3 days

Surgery Visit ± 2 days

Post Surgery Visit ± 2 days

Visit 2 ± 3 days

Visit 3 T#1 ± 3 days

Visit 4 ± 5 days

Visit every 6 months during continuation segment ± 15 days

Visit T#2 ± 5 days

Unscheduled Visits ± 3 days

Blood sample withdrawal every 2 months for immunogenicity test ± 15 days

# 14 RETENTION OF RECORDS

The Investigator is responsible for maintaining all records pertaining to the clinical trial and for ensuring complete and accurate documentation. The Investigator is, specifically, responsible for maintaining a subject identification log. This confidential subject identification code provides the link between named subject source records in the subject file and data provided to Kedrion S.p.A.

The Sponsor requires that Investigators at US sites retain records (all regulatory documents such as the protocol, study approval letters, all eCRFs, drug dispensing and accountability logs, all original subject consent forms and all correspondence pertaining to the conduct of the study) for a period of no less than 2 years from the date of final regulatory approval. If the study is discontinued, or if no application/license is to be filed or if the application/license is not approved for such indication, records should be retained for 2 years after the investigation is discontinued.

ICH Topic E6 (R1) Guidelines for GCP (CPMP/ICH/135/95) and the Directive 2003/63/EC (that modifies the Direcetive 2001/83/EC) instruct the investigators to keep the eCRF, the original documentation and any other support documentation for a minimum of 5 years.

Nevertheless, Kedrion requires to maintain the essential documents for a period of at least 15 years after the end/interruption of the study.

It is prohibited for study documents to be destroyed without prior written agreement between the Investigator and Kedrion S.p.A. If the Investigator wishes to assign the study records to another party, or move them to another location, Kedrion S.p.A. must be notified in writing, preferably before but not later than 10 days after the transfer.

# 15 PROPERTY OF DATA AND PUBLICATION

In collaboration with Kedrion S.p.A., ARG will prepare a draft study report after the completion of the study. The final draft study report may be sent to the selected Investigators for information, review, or comments.

Publication of data generated in the study is governed by the Investigator Clinical Trial Agreement. The investigator is informed that the data produced in this study will be used by the Sponsor in relationship with product development and, therefore, may be widespread to the Governmental agencies in various countries. To enable the use of data, the investigator should supply the Sponsor with the complete results of the tests, all the study data and all the study documents and the access to all the study documents. Any data analyses carried out independently by the PI should be submitted to Kedrion S.p.A. before publication or presentation for approval and permission.

Kedrion S.p.A. acknowledges the importance of the diffusion of medical data and, therefore, encourages their publication in reputed scientific journals and the diffusion during seminars and conferences.

Any result of the medical research on Kedrion S.p.A. products and/or the publication/oral diffusion/relevant manuscripts will be examined and discussed by the investigator and Kedrion S.p.A. representative 60 days before the submission for the publication or presentation during a conference. Due diligence should be exercised with the legitimate interests of Kedrion S.p.A., the paternity of the manuscript, obtaining patent protection, coordinating and keeping the exclusive nature of the submission to the health authorities, coordinating the other studies ongoing in the same area and protecting the confidential data and information.

Kedrion S.p.A. should receive one copy of each publication proposed. The comments of Kedrion S.p.A. should be issued without an exceeding delay and not beyond 60 days. In case of publication or presentation of the material of multicenter clinical trials, Kedrion S.p.A. should act as a coordinator and referee. The individual investigators of the multicenter trial cannot publish or submit data which are considered to be common to such a study without the consent of the other investigators and the previous review of Kedrion S.p.A.

In case of disagreement between the participants in a multicenter trial, Kedrion S.p.A. will be the final arbiter. The comments of Kedrion S.p.A. should be issued with no extra delay. Whenever they are not accepted, the senior author of the manuscript and the representatives of Kedrion S.p.A. will meet to discuss and find a common position for the final written text and/or the character of the publication. The above mentioned procedure also applies to data pertaining to studies prematurely discontinued or not completed.

The results of the investigators can be divulged to third parties from the trial team only with the paper version, as it was cleared. Kedrion S.p.A. will not mention the data of publications and investigators in its scientific information and/or promotional material without the complete acknowledgment of the sources (namely author and references).

# 16 COMPENSATION, LIABILITIES AND INSURANCE

Information on compensation, insurance, and indemnity will be supplied to the Investigator in the clinical study agreement. The Sponsor, Kedrion S.p.A., will pay for all study related costs. A separate financial agreement will be made (as appropriate) with the Investigator and/or Institutions.

In accordance with Legislative Decree 211/2003 and Decree of *Ministero del Lavoro, della Salute e delle Politiche Sociali* dated 14$^{th}$ July 2009 and its following modifications, Kedrion S.p.A. carries insurance coverage for incidents of damage or injury to study subjects while participating in the study or taking IMP. This coverage does not, however, cover any expenses that exceed the maximum premium and only covers the damages that are claimed during the period of validity in the policy. Such limitation does not alter the rights of the damaged subject to obtain compensation from the responsible party for any eventual damage.

The Investigator should inform the subjects participating to the clinical study of the insurance policy.

# 17 PROTOCOL AMENDMENTS

Only the Sponsor is authorized to modify the protocol. Changes to the protocol may be performed only after consultation and agreement with the Sponsor and the investigator.

The single exception is when the investigator deems that the subject's safety may be impaired without immediate action. In such cases, protocol amendments must be submitted for information or consideration to the applicable regulatory agencies. In EU, specifically, the immediate approval of the President of the EC should be required and the investigator should inform the Sponsor and the whole EC within 5 workings days after the occurrence of the emergence. IRB/EC approval will be requested for any change to this protocol which could affect the safety of subjects, the scope or design of the study.

A non-substantial amendment of a study protocol includes minor corrections or clarifications that have no significant impact on the way the clinical study is to be conducted and no effect on subject safety (e.g., administrative changes like change of telephone numbers, logistical changes, etc.). Minor procedural changes will be implemented by Study Notes to File (NTF), with supporting documentation at each site, if appropriate. Changes to Appendices are not subject to IRB/EC approvals, but are documented via Notes to File.

Changes to study personnel or representatives, study facilities (e.g. sites, central laboratories) and/or procedures for handling the samples do not constitute a protocol amendment, but will only require changes in the appropriate appendices.

Any changes of the protocol (substantial amendments and non-substantial amendments) will be integrated into an updated study protocol, with a listing of all changes and reasoning for them.

# 18 REFERENCES

Caputo R, Neri P *et al.* Long-term efficacy of surgical removal of pseudomembranes in a child with ligneous conjunctivitis treated with plasminogen eye drops. Thromb Haemost. 2008; 100: 1196–1198.

CHMP/EWP/83561/2005, 27 July 2006. Guideline on Clinical Trials in Small Populations.

CPMP/SWP/2145/00, 1 March 2001. Note for Guidance on Non-Clinical Local Tolerance Testing of Medicinal Products.

CPMP/ICH/2711/99, February 2001. Note for Guidance on Clinical Investigation of Medicinal Products in the Paediatric Population.

CPMP/ICH/135/95, July 2002. ICH Topic E6 (R1). Guideline for Good Clinical Practice.

CPMP/SWP/2145/00, 1 March 2001. Note for Guidance on Non-Clinical Local Tolerance Testing of Medicinal Products.

Dir. 2001/20/EC of the European Parliament and the Council. 4 April 2001. The approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use.

Dir. 2001/83/EC of the European Parliament and the Council. 6 November 2001. The Community code relating to medicinal products for human use.

Chtourou S, Porte P, Bihoreau N *et al.* A solvent/detergent-treated and 15-nm filtered factor VIII a new safety standard for plasma-derived coagulation factor concentrates. Vox Sanguinis. 2007; 92:327-337.

Drew AF, Kaufman AH, Kombrinck KW *et al.* Ligneous conjunctivitis in plasminogen-deficient mice. Blood.1998; 91(5):1616-1624.

Heidmann DG, Williams GA, Hartzer M *et al.* Treatment of ligneous conjunctivitis with topical plasmin and topical plasminogen. Cornea. 2003; 22(8):760-762.

Kao WW, Kao CW, Kaufman AH *et al.* Healing of corneal epithelial defects in plasminogen- and fibrinogen-deficent mice. IOVS.1998; 39(3):502-508.

Legislative Decree no.211 of June 24, 2003: “Transposition of Directive 2001/20/EC relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for clinical use”

Mingers AM, Heimburger N, Lutz E. Familiärer homozygoter und heterozygoter Typ I-Plasmonigenmangel. Scharrer I, Schramm W (eds). 25. Hämophilie-Symposium 1994. Springer, Berlin, 1995: 96-104.

Ministerial Decree of May 10, 2001: “Controlled clinical trials conducted by General Practitioners and Paediatricians”.

Ministerial Decree of 15 July 1997: “Transposition of guidelines of the European Union in good clinical practice for the conduction of clinical trials with medicines”.

Ministerial Decree of 14 July 2009: “Minimum requirements for insurance policies which safeguard participants to clinical trials of medicinal products”.

Schott D, Dempfle CE, Beck P *et al.* Therapy with a purified plasminogen concentrate in an infant with ligneous conjunctivitis and homozygous plasminogen deficiency.1998; 339(23):1679-1686.

Schuster V, Mingers AM *et al.* Homozygous mutations in the plasminogen gene of two unrelated girls with ligneous conjunctivitis.1997; 90:958-966.

Schuster V, Seidenspinner S, Zeitler P *et al.* Compound-heterozygous mutations in the plasminogen gene predispose to the development of ligneous conjunctivitis. Blood 1999; 93(10):3457-3466.

Schuster V, Zeitler P, Seregard S *et al.* Homozygous and compound-heterozygous Type I Plasminogen Deficiency is a common of ligneous conjunctivitis. Thromb Haemost.2001; 85:1004-10.

Schuster V, Seregard S. Ligneous Conjunctivitis. Survey of Ophtalmology.2003;48(4):369-388.

Siboni SM, Spreafico M, *et al.* Molecular characterization of an Italian Subject with plasminogen deficiency and ligneous conjunctivitis. Blood Coagul Fibrinolysis 2007; 18: 81–4.

Tefs K, Tait CR,*et al.* A K19E missense mutation in the plasminogen gene is a common cause of familial hypoplasminogenaemia. Blood Coagul Fibrinolysis 2003; 14: 411–6.

Tefs K, Gueorguieva M, Klammt J *et al.* Molecular and clinical spectrum of type I plasminogen deficiency: a series of 50 Subjects. Blood.2006; 108(9):3021-3026.

Watts P, Suresh P, Mezer E *et al.* Effective treatment of ligneous conjunctivitis with topical plasminogen. Am J Ophthalmol.2002; 133(4):451-454.

# 19 APPENDICES

## 19.1 Appendix 1 – Plasminogen Molecular Analysis

The molecular analysis of plasminogen will be performed to identify and study genetic mutations in the gene coding for plasminogen that may have caused Type 1 plasminogen deficiency.

**DNA EXTRACTION**

The molecular analysis is performed starting from the extraction of genomic DNA from peripheral blood leukocytes [redacted]. DNA was isolated from blood cells according to the salting out method (Miller SA et al. *Nucleic Acids Res 1988;16:1215*)

**PCR AMPLIFICATION**

[redacted]

**SEQUENCING AND ANALYSIS**

[redacted]

## 19.2 Appendix 2 – Vendors List*

*CRO*:

- ✓ **ATLANTIC RESEARCH GROUP, INC.**, 2421 Ivy Road, Suite 200, Charlottesville, VA 22903
  - ➢ Study Management

*Centralized Laboratories*:

- ✓ **LabConnect, LLC**[(1)] 2304 Silverdale Road, Suite 100 Johnson City, TN 37601
  - ➢ Immunogenicity: **Machaon Diagnostics**[(2)] 3023 Summit Street Oakland, CA 94609
  - ➢ Hematology, Chemistry and Viral Serology for HIV1-2, HCV, HAV and HBV[(3)]: **Mountain States Health Alliance Johnson City Medical Center** [(2)] 400 N. State of Franklin Rd. Johnson City, TN 37604
  - ➢ NAT for HIV1-2, HCV, HBV, Parvovirus B19; Viral Serology for Parvovirus B19[(3)]: **Quest Diagnostics Nichols institute**[(2)] 14225 Newbrook Drive Chantilly, VA 20151
  - ➢ HAV NAT[(3)]:**Qualtex Laboratories**[(2)] 6211 IH 10 W San Antonio, Texas 78201
- ✓ **Eurofins Biolab Srl** via Bruno Buozzi 2 – 20090 Vimodrone (MI).
  - ➢ Plasminogen activity and antigen testing
- ✓ **Laboratorio di Patologia Molecolare applicata alla clinica** - Fondazione Luigi Villa, Centro Emofilia e Trombosi Ospedale Maggiore di Milano Via Pace, 9 - 20122 Milano
  - ➢ Genetic Testing
- ✓ **Covance Labs, Inc.** 3635 Concorde Parkway, Suite #100 Chantilly, VA 20151
  - ➢ Immunogenicity

*Centralized Warehouse*

- ✓ **Fisher Bioservices**[(1)(4)] 14665 Rothgeb Drive, Rockville, MD 20850
  - ➢ IMP Storage and management

*Home Health Agency*

- ✓ **PCM Trials** 1600 Emerson Street, Denver, CO 80218
  - ➢ Home Health Service

[(1)] ARG, INC Contractors
[(2)] LAB Connect, LLC Contractors
[(3)] Only for US site. For Italy sites, these test will be performed by local site laboratory
[(4)] Only for US site. For Italy sites, the IMP Storage and Management is under Sponsor responsibility

*The list is referred to all vendors currently involved or that were involved in the clinical trial.

## 19.3 Appendix 3 – The Declaration of Helsinki

# WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964
and
amended by
the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

## Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

   The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

## General Principles

3. The Declaration of Geneva of the WMA binds the physician with words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and e□ects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12 . Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely a□ect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

### Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

## Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specificaly considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

## Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to general y accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

## Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as

applicable international norms and standards but these must not be al owed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especial y information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

## Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

## Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional a□liations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formal y documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legal y authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legal y authorised representative. The potential subject's dissent should be respected.
30. Research involving subjects who are physical y or mental y incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legal y authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legal y authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely a□ect the patient- physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

## Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less e□ective than the best proven one, the use of placebo, or no intervention is necessary to determine the e□cacy or safety of an intervention

and the patients who receive any intervention less e□ective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

## Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

## Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers al have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. Al parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

## Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legal y authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.