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CLINICAL STUDY PROTOCOL

A randomized, active-controlled, parallel, double-blind study on the safety, ocular tolerability and efficacy of Piiloset Trehalose Emulsion Eye Drop in adult patients with moderate or severe dry eye

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SYNOPSIS

Sponsor: Oy Finnsusp Ab	**CLINICAL STUDY PROTOCOL** **"Piiloset-emulsiotippatutkimus"**
Name of the investigational device: Piiloset Trehalose Emulsion Eye Drop (a working title)	
Study title: A randomized, active-controlled, parallel, double-blind study on the safety, ocular tolerability and efficacy of Piiloset Trehalose Emulsion Eye Drop in adult patients with moderate or severe dry eye	
Investigator and study centre: Kai Kaarniranta, MD, PhD, Professor Kuopio University Hospital, Department of Ophthalmology, Puijonlaaksontie 2, 70200 Kuopio, Finland	
Objectives: Primary objectives **Part 1**. To evaluate the safety and ocular tolerability of trehalose emulsion eye drops administered on one eye four times a day for one day. **Part 2.** To evaluate the safety, ocular tolerability and efficacy (OSDI, tear osmolarity, tear film break-up time) of trehalose emulsion eye drops administered on one eye three times a day for 10 (± 1) days as a change from baseline and compared to control eye drops. **Part 3.** To evaluate the safety, ocular tolerability and efficacy (OSDI, tear osmolarity, tear film break-up time) of trehalose emulsion eye drops administered on both eyes three times a day for 30 (± 2) days as a change from baseline and compared to control eye drops. Secondary objectives: **Part 2.** To evaluate the efficacy (blink rate, corneal and conjunctival staining) of trehalose emulsion eye drops administered on one eye three times a day for 10 (± 1) days as a change from baseline and compared to control eye drops. **Part 3.** To evaluate the efficacy (blink rate, corneal and conjunctival staining) of trehalose emulsion eye drops administered on both eyes three times a day for 30 (± 2) days as a change from baseline and compared to control eye drops.	
Methodology: This is a prospective, single-site, randomized, active-controlled, parallel, double-blind (Parts 2 and 3), and multiple dose study to evaluate the safety, ocular tolerability and efficacy of Piiloset Trehalose Emulsion Eye Drop in the adult patient population with moderate or severe dry eye for up to 30 days.	
Sample size: **Part 1.** Three participants (at least one male and one female) will be included for treatment. **Part 2.** Nine participants (both males and females) will be included for treatment to provide data from at least 8 subjects.	

Part 3. Fifty-two participants (both males and females) will be included for treatment to provide data from at least 46 subjects.

No subject will be allowed to participate in more than one Part of the study.

Criteria for eligibility:

Inclusion criteria

1. Ability and willingness to give informed written consent prior to any screening procedure after explanation of the nature and possible consequences of the study.
2. Age between 18 and 80 years.
3. At least two of the following conditions (A and B):
   A. Symptomatic dry eye with OSDI score ≥20. AND
   B1. Tear film break-up time (TBUT) <10 seconds. OR
   B2. Positive ocular (corneal and conjunctival) staining pattern by Oxford grading scale.
4. Body weight at least 45 kg.
5. Under stable topical and/or systemic therapy for at least 4 weeks before the study procedures and apparent ability and willingness to abstain from other therapies until completion of the study period.
6. Ability and willingness to self-administer eye drops.
7. Ability and willingness to understand and fill in the OSDI questionnaire.
8. Ability and willingness to comply with the study protocol and other study-related procedures.

Exclusion criteria

1. History of ocular surgery, trauma, or refractive laser vision correction procedure less than 3 months earlier.
2. Evidence of acute or chronic infection in the cornea or conjunctiva.
3. Diagnosis of Sjögren's syndrome.
4. Unwillingness or apparent disability to discontinue contact lens use during study period and at least one week before the first dosing day.
5. Current ocular allergy symptoms.
6. Known allergy to any constituent of the trehalose emulsion eye drops (trometamol, citric acid, sodium hyaluronate, trehalose, glycerol, sacha inchi seed oil, Tween 80, or Span 80) or control eye drops (sodium hyaluronate).
7. Currently pregnant, nursing or planning to become pregnant before completion of the study period.
8. Any other condition that may, in the Investigator's opinion, jeopardize the safety or availability of the subject or adherence to the study protocol or may interfere with the interpretation of the results and would thus make the subject inappropriate for entry in the study.

Investigational device:

Piiloset Trehalose Emulsion Eye Drop is an eye care product in the form of eye drops to be administered on the eye. [deleted]

Control product: The control device is an [deleted] eye drop containing 0.2% medium-molecular-weight hyaluronic acid. The product is a CE-marked medical device. The product is used in Parts 2 and 3.
Duration of treatment: **Part 1.** Four drops of Piiloset Trehalose Emulsion Eye Drop at approximately 2-hour intervals on the worst eye during office hours of one day at the clinic **Part 2.** One drop 3 times a day on a randomized eye for 10 (± 1) days at home **Part 3.** One drop of the randomized product 3 times a day on both eyes for 30 (± 2) days at home
Assessments: Safety and ocular tolerability: Physical examination of the eyes will be performed by the Investigator at screening, in Part 1 at baseline and after the 4th dose, in Parts 2 and 3 at baseline and after the last dose. The Investigator will also evaluate possible local eye reactions visually at visits in all Parts. Ocular comfort rating using the OSDI questionnaire will be performed at screening, before the 1st treatment in all Parts, and at end-of-study visit in Parts 2 and 3. AEs will be collected throughout the study. Efficacy: OSDI, tear osmolarity, TBUT, blink rate, and corneal and conjunctival staining will be measured at screening, before the 1st treatment in all Parts, and at end-of-study visit in Parts 2 and 3.
Statistical methods: Descriptive statistics by treatment group and visit day will be provided to summarize the results. Also changes from baseline will be summarized with descriptive statistics. No imputations will be done for missing observations. If not stated otherwise, a two-sided p-value less than 0.05 will be considered statistically significant. Changes in OSDI scores in Part 3 will be compared by treatment groups using analysis of variance model. No interim analyses are planned.

ABBREVIATIONS AND DEFINITION OF TERMS

ADE	adverse device effect
AE	adverse event
CRF	case report form
D	day
EC	ethics committee
FDA	Food and Drug Administration
GCP	good clinical practice
ETDRS	early treatment diabetic retinopathy study
IB	investigator's brochure
IBI	interblink interval
IER	Institute of Eye Research
IOP	intraocular pressure
ITF	investigator's trial file
OPI	ocular protection index
OSDI	ocular surface disease index
ITT	intent-to-treat
PP	per-protocol
SADE	serious adverse device effect
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
TBUT	tear film break-up time
TFOS DEWS II	Tear Film and Ocular Surface Society's Dry Eye Workshop II
USADE	unanticipated serious adverse device effect

1. INTRODUCTION

[deleted]

2. STUDY OBJECTIVES

2.1. Primary objectives

Part 1. To evaluate the safety and ocular tolerability of trehalose emulsion eye drops administered on one eye four times a day for one day.

Part 2. To evaluate the safety, ocular tolerability and efficacy (OSDI, tear osmolarity, tear film break-up time) of trehalose emulsion eye drops administered on one eye three times a day for 10 days as a change from baseline and compared to control eye drops.

Part 3. To evaluate the safety, ocular tolerability and efficacy (OSDI, tear osmolarity, tear film break-up time) of trehalose emulsion eye drops administered on both eyes three times a day for 30 days as a change from baseline and compared to control eye drops.

2.2. Secondary objectives

Part 2. To evaluate the efficacy (blink rate, corneal and conjunctival staining) of trehalose emulsion eye drops administered on one eye three times a day for 10 days as a change from baseline and compared to control eye drops.

Part 3. To evaluate the efficacy (blink rate, corneal and conjunctival staining) of trehalose emulsion eye drops administered on both eyes three times a day for 30 days as a change from baseline and compared to control eye drops.

Assessments to be performed for each objective are described in chapter 6.

3. STUDY DESIGN

3.1. Type and design of the study

This is a prospective, single-site, randomized, active-controlled, parallel, double-blind, single and multiple dose study to evaluate the safety, ocular tolerability and efficacy of Piiloset Trehalose Emulsion Eye Drop in the adult patient population with moderate or severe dry eye for up to 30 days.

Part 1. Three subjects (n=3) are dosed with four doses (approximately 2 hours apart) of the emulsion eye drops on the worst eye during office hours of one day at the clinic. The second and third doses will be administered if no signs of significant irritation or other adverse device effect (ADEs) after previous doses are observed by the Investigator.

In the absence of significant ADEs in all patients after the last dose, as assessed by the Investigator, Part 2 will commence.

Part 2. Nine subjects (n=9) self-administer the emulsion eye drops on a randomized eye and the control eye drops on the contralateral eye three times a day for a total of 10 (± 1) days. The efficacy end points including

OSDI, tear osmolarity, blink rate, corneal and conjunctival staining, and TBUT will be assessed at baseline (Day 1) and after the last dose (Day 10). The safety and tolerability in both eyes will be evaluated. The patient and the study personnel are blinded to the identity of the study products.

In the absence of significant ADEs in all patients after the last dose, as assessed by the Investigator, Part 3 will commence.

Part 3. Out of 52 subjects, 26 randomized subjects self-administer the emulsion eye drops and 26 subjects apply the control eye drops on both eyes three times a day for a total of 30 (± 2) days in a double-blinded fashion. The efficacy end points including OSDI, tear osmolarity, blink rate, corneal and conjunctival staining, and TBUT will be assessed at baseline (Day 1) and after the last dose (Day 30). The safety and tolerability in both eyes will be evaluated.

Adverse events (AEs) will be collected throughout the study. The Investigator will perform the ophthalmologic assessments and will be available for consultation throughout the study.

3.2. General study outline

A schematic presentation of the study events with particular procedures to be performed on the corresponding study day in each Part is provided in Table 2.

Table 2. Study events

Study event (chapter number)	Screening	Part 1	Part 2		Part 3	
		Day 1	Day 1	Day 10	Day 1	Day 30
Informed consent (4.3)	x					
Eligibility criteria (4.2)	x					
Baseline characteristics (6)	x	x	x		x	
Safety and ocular tolerability assessments (6.3.1)	x	x	x	x	x	x
Adverse events (6.3.1)	x	x	x	x	x	x
Efficacy assessments (6.3.2, 6.4.2)	x	x	x	x	x	x
Dosing at study site (non-randomized, unblinded; 3.2.3, 5.2)		x				
Dosing at home (randomized, double-blind; 3.2.3, 5.3, 5.4)			x	x[1]	x	x[1]
Diary filling (4.6, 5.5, 5.8, 6.3.1)			x	x[1]	x	x[1]

[1] Including study days after Day 1.

3.2.1. Randomization and blinding

Part 1. No randomization or blinding will be used.

Part 2. The randomized test item will be administered on a randomized eye of the subject in a double-blinded fashion. The randomization will be balanced for the right and left eyes.

Part 3. The test item will be administered on both eyes of the randomized subject in a double-blinded fashion. The randomization will be balanced for the gender of the subjects.

Randomization lists will be prepared and stored at 4Pharma. The code envelope will be opened prematurely on the Investigator's resolution in cases of emergency.

3.2.2. Screening

The screening visit will be performed within two weeks before the first dosing day (Day 1). A prospective subject will receive both written and verbal information of the study, an opportunity to ask questions, and sufficient time to consider the decision to participate in the study. Written informed consent will be obtained if the subject decides to participate.

The medical history of the subjects will be recorded by using questionnaire. Physical examination of the eyes, digital photography of the anterior parts of the eyes, and screening assessments including OSDI, tear film break-up time (TBUT), and corneal and conjunctival staining are performed by the Investigator. Subjects are given a study diary to document daily administration of the study treatments, possible AEs, concomitant medications, and any deviations from the instructions given. The diaries are checked and collected at the last visit.

The subjects are included in the study after the results of the screening assessments are available. The Study Nurse will contact the subject and give detailed information on the visit schedule.

3.2.3. Study procedures

The study comprises three Parts with scheduled visits on Day 1 (all Parts) and on end-of-study date (Parts 2 and 3) to the study centre. Each subject will participate in no more than one Part of the study. AEs will be monitored and recorded throughout the study.

Part 1. The subjects arrive at the study centre at an agreed time in the morning. Physical examination of the eyes and digital photography of the anterior parts of the eyes will be performed. Baseline eye assessments will be performed. The subjects will be dosed topically with a daily total number of four drops of Piiloset Trehalose Emulsion Eye Drop in an open-label manner at approximately 2-hour intervals on the worst eye, while the other eye serves as an untreated control. Tolerability assessment of the eye is performed before the next dosing. If there are no signs of irritation or other treatment-emergent AEs, the next doses will be administered and the subjects will be followed at the study site until one hour after the last dose, at which time point the physical examination of the eye will be performed again. If there are no safety concerns, the subjects may leave the study centre. The participants are not allowed to enter Parts 2 or 3.

Part 2. The subjects arrive at the study centre at an agreed time in the morning on Day 1. Physical examination of the eyes, digital photography of the anterior parts of the eyes, and baseline assessments including OSDI, tear osmolarity, blink rate, corneal and conjunctival staining, and TBUT are performed. The subjects self-administer one drop of Piiloset Trehalose Emulsion Eye Drop on a randomized eye and one drop of the control eye drops on the contralateral eye under control of the Study Nurse. The subjects then receive the treatment eye drop bottles to themselves with detailed instructions on how to continue dosing at home three times a day for the next 10 (± 1) days, including Day 1. The subjects are urged to contact the study personnel in case of an AE, especially in the ocular area before Day 10. They will be invited to visit the study site when clinically indicated, based on the judgement of the Investigator. The subjects may then leave the study centre.

The subjects arrive at the study centre on Day 10 (± 1) for an end-of-study visit. Only the morning dose will be administered on the same day. Physical examination of the eyes, digital photography of the anterior parts of the eyes, and ocular assessments including OSDI, tear osmolarity, blink rate, corneal and conjunctival staining, and TBUT are performed. In addition, the subjects are asked to return the used eye drop bottles. The study diaries are collected, checked, and marked on case report forms (CRFs).

Possible AEs and other study-related issues are monitored and discussed with the subjects and recorded. Any abnormal findings will be assessed according to their clinical importance and, if found to be possibly clinically significant but not study-related, the subject is instructed to contact his/her own health care provider.

The participants are not allowed to enter Part 3. Possible discontinued subjects will be invited to an end-of-study visit which should be performed within one week after the date of discontinuation, if possible.

Part 3. The subjects arrive at the study centre at an agreed time in the morning on Day 1. Physical examination of the eyes, digital photography of the anterior parts of the eyes, and baseline assessments including OSDI, tear osmolarity, blink rate, corneal and conjunctival staining, and TBUT are performed. The subjects self-administer one drop of the randomized treatment eye drop (either Piiloset Trehalose Emulsion Eye Drop or the control eye drops) on both eyes under control of the Study Nurse. The subjects then receive the treatment eye drop bottle to themselves with detailed instructions on how to continue dosing at home three times a day for the next 30 (± 2) days, including Day 1. The subjects are urged to contact the study personnel in case of an AE, especially in the ocular area before Day 30. They will be invited to visit the study site when clinically indicated, based on the judgement of the Investigator. The subjects may then leave the study centre.

The subjects arrive at the study centre on Day 30 (± 2) for an end-of-study visit. Only the morning dose will be administered on the same day. Physical examination of the eyes, digital photography of the anterior parts of the eyes, and ocular assessments including OSDI, tear osmolarity, blink rate, corneal and conjunctival staining, and TBUT are performed. In addition, the subjects are asked to return the used eye drop bottle. The study diaries are collected, checked, and marked on CRFs.

Possible AEs and other study-related issues are monitored and discussed with the subjects and recorded. Any abnormal findings will be assessed according to their clinical importance and, if found to be possibly clinically significant but not study-related, the subject is instructed to contact his/her own health care provider.

Discontinuation. Possible discontinued subjects will be invited to an end-of-study visit which should be performed within one week after the date of discontinuation, if possible. Physical examination of the eyes and digital photography of the anterior eye are performed. In addition, the subjects are asked to return the used eye drop bottle. The study diaries are collected, checked, and marked on CRFs. Possible AEs and other study-related issues are monitored and discussed with the subjects, and recorded.

4. SUBJECTS

4.1. Source population

The source population to be recruited in the study will consist of outpatients with a diagnosis of dry eye, evaluated at the Kuopio University Hospital (Kuopio, Finland).

4.2. Eligibility requirements

4.2.1. Inclusion criteria

Subjects to be included in the study have to fulfil the following criteria:

1. Ability and willingness to give informed written consent prior to any screening procedure after explanation of the nature and possible consequences of the study.
2. Age between 18 and 80 years.
3. At least two the following conditions (A and B):
   A. Symptomatic dry eye with OSDI score ≥20. (Wolffsohn et al. 2017) AND
   B1. Tear film break-up time (TBUT) <10 seconds. (Wolffsohn et al. 2017) OR
   B2. Positive ocular (corneal and conjunctival) staining pattern by Oxford grading scale.
4. Body weight at least 45 kg.
5. Under stable topical and/or systemic therapy for at least 4 weeks before the study procedures and apparent ability and willingness to abstain from other therapies until completion of the study period.
6. Ability and willingness to self-administer eye drops.
7. Ability and willingness to understand and fill in the OSDI questionnaire.
8. Ability and willingness to comply with the study protocol and other study-related procedures.

4.2.2. Exclusion criteria

Subjects will not be included in the study if they fulfil any of the following criteria:

1. History of ocular surgery, trauma, or refractive laser vision correction procedure less than 3 months earlier.
2. Evidence of acute or chronic infection in the cornea or conjunctiva.
3. Diagnosis of Sjögren's syndrome.
4. Unwillingness or apparent disability to discontinue contact lens use during study period and at least one week before the first dosing day.
5. Current ocular allergy symptoms.

6. Known allergy to any constituent of the trehalose emulsion eye drops (trometamol, citric acid, sodium hyaluronate, trehalose, glycerol, sacha inchi seed oil, Tween 80, or Span 80) or control eye drops (sodium hyaluronate).
7. Currently pregnant, nursing or planning to become pregnant before completion of the study period.
8. Any other condition that may, in the Investigator's opinion, jeopardize the safety or availability of the subject or adherence to the study protocol or may interfere with the interpretation of the results and would thus make the subject inappropriate for entry in the study.

4.3. Recruitment and screening

The recruitment of subjects will start after obtaining a positive statement from The Research Ethics Committee of the Northern Savo Hospital District (EC) and after notifying Valvira of the clinical investigation.

During routine visits to the clinic, patients of potential suitability for the study are proposed to consider participating by the Study Nurse or the Investigator. The purpose and course of the study will be described in layman's language. If the patient might be suitable and willing for inclusion in the study, the subject information leaflet and the informed consent form are delivered to the patient in hand, by regular mail, e-mail or another suitable method. A screening visit to the clinic will be arranged.

At the screening visit, informed written consent will be obtained before any screening procedure. The inclusion and exclusion criteria will be checked and documented. If an inclusion criterion is not fulfilled or an exclusion criterion is fulfilled, this is immediately told to the subject and the interview is terminated with possible health-related instructions to the volunteer.

The study subjects are explained what personal information of them will be collected and how and where it will be stored and processed. The collection of personal information is described in the Description of the Personal Data File which is kept in the Investigator's Trial File (ITF) and made available to everyone.

4.4. Number of subjects

Part 1. Three participants (at least one male and one female) will be included for treatment.

Part 2. Nine participants (both males and females) will be included for treatment to provide data from at least 8 subjects.

Part 3. Fifty-two participants (both males and females) will be included for treatment to provide data from at least 46 subjects. (See sample size estimation, 7.1).

No subject will be allowed to participate in more than one Part of the study.

4.5. Assignment of treatments and subject study numbers

At the screening visit, the subject will be given a consecutive subject study number once the enrolment has been confirmed and the informed consent obtained.

Part 1. Piiloset Trehalose Emulsion Eye Drop will be administered on the worst eye of three subjects four times (approximately 2 hours apart) during one day.

Part 2. Piiloset Trehalose Emulsion Eye Drop will be self-administered on a randomized eye and the active control eye drops by 9 subjects on the contralateral eye three times a day and in a double-blinded fashion.

Part 3. Piiloset Trehalose Emulsion Eye Drop will be self-administered by 26 randomized subjects and the active control eye drops by 26 randomized subjects on both eyes three times a day for a total of 30 days in a double-blinded fashion.

4.6. Instructions concerning lifestyle and concomitant treatments

The subjects will be advised to comply with the following instructions applicable for Parts 2 and 3:

1. The subjects must report all AEs and concomitant treatments to the study personnel. Minor AEs may be reported at study visits and significant AEs as soon as possible.
2. At home, the study subjects are to fill in the diary (AEs, concomitant medications, other deviations, dose applications) starting from the first dosing until the end-of-study visit.
3. The subjects are instructed not to participate in another clinical study during the course of this study.
4. The subjects are reminded to use adequate contraceptive measures during the study, if applicable. (See exclusion criterion 7.)

4.7. Withdrawal of subjects

A subject is withdrawn from the study prematurely in the following conditions:

1. an AE will jeopardize the subject's well-being, as judged by the Investigator or the Sponsor;
2. the subject's adherence to the protocol is no longer acceptable;
3. the medical condition of the subject deteriorates between screening and the first study treatment in a way that causes the inclusion criteria being no longer fulfilled; or
4. the subject wishes to discontinue/to be withdrawn at any time.

Any subject who is withdrawn before the first study treatment dose on Day 1 will be replaced, if possible. Later withdrawals will not be replaced. All data collected from the subject until withdrawal will be included in the study file. A withdrawn/discontinuing subject is not allowed to re-enter the study later. The Sponsor must be notified of premature discontinuations without delay.

4.8. Payments and compensation to subjects

The subjects are not paid for participation in the study. A compensation for direct costs such as travel expenses or loss of wages may be paid to the subject or his/her representative according to regulations of The Ministry of Social Affairs and Health of Finland.

5. INVESTIGATIONAL TREATMENTS

5.1. Investigational medical device and control device

The investigational medical device is Piiloset Trehalose Emulsion Eye Drop (a working title). It is an eye care product in the form of eye drops to be administered on the eye.

[deleted]

The control device is [deleted] eye drop containing 0.2% medium-molecular-weight hyaluronic acid [deleted]. The product is a CE-marked [deleted] medical device. The product is used in Parts 2 and 3.

Both investigational devices are sterile in visually similar 10-ml squeezable multidose primary packages designed for preservative-free eye drop products, giving no indication of the identity of the product. Each vial is labelled with a unique code. The manufacturer of both devices is Oy Finnsusp Ab, Pääskykalliontie 5, FI-21420 Lieto, Finland (the Sponsor).

5.2. Treatments in Part 1

The subjects will be dosed topically with a daily total of four drops of Piiloset Trehalose Emulsion Eye Drop in an open-label manner at approximately 2-hour intervals on the worst eye, while the other eye serves as an untreated control. The subjects will be followed until one hour after the last administration at the study site.

5.3. Treatments in Part 2

If no significant ADEs are observed by the Investigator in Part 1, the subjects enrolled in Part 2 will receive one dose of both the emulsion eye drops and the control eye drops. The subjects self-administer the emulsion eye drops on a randomized eye and the control eye drops on the contralateral eye three times a day for a total of 10 days. The investigational medical device and the control device are coded to ensure blinding to the identity of the study products. It will be marked on the label for each subject individually to which eye the product is to be used.

5.4. Treatments in Part 3

If no significant ADEs are observed by the Investigator in Part 2, the subjects enrolled in Part 3 will receive one dose of either the emulsion eye drops or the control eye drops. The subjects self-administer the eye drops on a both eyes three times a day for a total of 30 days. Each investigational device is coded to ensure blinding to the identity of the study products.

5.5. Handling of the investigational devices

At the study site, the investigational devices are to be kept at ambient room temperature (15–25 °C) in a locked cabinet. The Investigator will maintain a study drug accountability list. Each bottle will be weighed by the Study Nurse before giving it to the subject. At home, the investigational devices should be stored at ambient room temperature out of the reach of children. The opened eye drop bottles can be used up to the end-of-study visit of each Part. At the last visit of parts 2 and 3, the bottles are to be returned to the study

site. The subjects will record the self-administration time (date, approximate clock time, and possible comments) of the investigational devices in a study diary.

The investigational devices in Parts 2 and 3 are to be used in the normal way the subject would use any eye drop product. No specific instructions for hygiene of the hands, eyes, or storage of the bottles etc. will be given.

5.6. Monitoring of the conduct of the trial

The study is to be conducted in a hospital environment with established study procedures, assessments, and collection of information. The Investigator is experienced with randomized clinical trials. No external monitoring is implemented. The quality assurance personnel of the Kuopio University Hospital and the competent authorities may conduct audits in any phase of the study.

5.7. Monitoring of trial adherence

The study personnel will administer all doses in Part 1 to ensure 100% compliance (adherence).

In Parts 2 and 3, the first dose is self-administered under the control of the study personnel. Adherence to the dosing schedule thereafter will be followed by asking the subjects at the end-of-study visit and in their study diaries. The used bottles collected at the end-of-study visit will be weighed by the Study Nurse. The used amount of the investigational device will be compared to calculated expected use to evaluate the level of adherence. The intuitive design and handling of the bottle is expected to support patient adherence.

5.8. Concomitant treatments

The subjects are instructed to record in their study diary all concomitant medications starting from the first dosing day until the end-of-study visit.

6. ASSESSMENTS

6.1. Screening data

Demographic and other baseline information to be recorded on CRFs during the screening visit includes the following:

- age
- gender
- weight
- history of ocular surgery, trauma, refractive laser vision correction, infection, allergy, or Sjögren's syndrome
- topical and/or systemic therapies for the preceding 4 weeks
- allergy to any constituent of the investigational
- current or planned pregnancy and nursing

6.2. Methods for physical examination of the eyes

For safety, ocular tolerability, and efficacy endpoints, the Investigator will perform the physical examination of the eyes. The examination includes several methods which will serve as indicators for selected endpoints as explained below.

Physical examination of the eyes includes the following methods:

- visual acuity (ETDRS)
- blink rate
- tear osmolarity ([deleted]
- measurement in tear sample)
- digital photography of the anterior eye
- tear film break-up time (TBUT under biomicroscope)
- corneal staining (Oxford grading under biomicroscope)
- conjunctival staining (Oxford grading under biomicroscope)
- bulbar redness (IER grading under biomicroscope)
- lid redness (IER grading under biomicroscope)
- intraocular pressure (IOP)

6.3. Assessments for primary objectives

6.3.1. Safety and ocular tolerability

The safety and ocular tolerability of trehalose emulsion eye drops administered on the eyes will be evaluated using the following assessments.

A. Physical examination of the eyes

Physical examination of the eyes will be performed

- at screening;
- in Part 1 at baseline (before the 1st dose) and after the 4th dose;
- in Part 2 at baseline (Day 1, before the 1st dose) and after the last dose; and
- in Part 3 at baseline (Day 1, before the 1st dose) and after the last dose.

Methods:

- visual acuity (ETDRS)
- digital photography of the anterior eye
- bulbar redness (IER grading)
- lid redness (IER grading)
- intraocular pressure (IOP)

The Investigator will also evaluate local eye reactions visually after each dosing in Part 1 and at visits in Parts 2 and 3.

B. OSDI

Ocular comfort rating for measuring the ocular tolerability of the investigational treatments will be performed using the OSDI questionnaire

- at screening visit;
- before the 1st treatment in all Parts; and
- at end-of-study visit in Parts 2 and 3.

C. Adverse events (AEs)

AEs will be collected throughout the study. When at the study site, the subjects are monitored for AEs and urged to report of AEs immediately when they appear. Outside the study visits, the subjects are asked to record all AEs in their study diary and to contact the study personnel in case of significant, particularly eye-related AEs; in case of emergency, the subjects are instructed to visit a physician at a health care centre or hospital and to contact the Investigator as soon as possible. The Investigator will be available on site (Part 1) or by phone (Parts 2 and 3) throughout the study for eye-related emergencies. If the clinical condition of the subject requires, a follow-up will be arranged. In case of minor AEs, the subjects are to report them on their next visit. In cases of illness during the study with no obvious connection to study participation, the subjects are instructed to seek regular medical care.

In a case of an SAE, the Investigator will contact the Sponsor as soon as possible. An initial SAE report will be submitted to the Sponsor as agreed between the parties, and a follow-up report will be provided later, if indicated.

The contact persons and address of the Sponsor for SAEs are:

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21420 Lieto

The definitions for ADE, AE, SADE, SAE, USADE and their documentation follow the EN ISO 14155 guidance.

Only if there are no safety concerns and no substantial changes in baseline since the screening visit, as deemed by the Investigator, the subject may continue his/her participation. Only in the absence of significant ADEs in all patients after the last dose in Parts 1 and 2, as deemed by the Investigator, the study will proceed to the next Part.

6.3.2. Efficacy

The efficacy of the investigational treatments on signs and symptoms of dry eye will be evaluated using the following assessments:

D. OSDI

The OSDI questionnaire comfort rating used as an efficacy endpoint will be performed

- before the 1st treatment in all Parts; and
- at end-of-study visit in Parts 2 and 3.

E. Biomarkers

Biomarkers to be assessed as primary efficacy endpoints will be determined

- at screening visit;
- before the 1st treatment in all Parts; and
- at end-of-study visit in Parts 2 and 3.

Biomarkers used as primary efficacy endpoints are a part of the physical examination of the eyes and include the following methods:

- tear osmolarity ([deleted])
- tear film break-up time (TBUT)

6.4. Assessments for secondary objectives

6.4.1. Safety and ocular tolerability

The safety and ocular tolerability assessments will be performed for the primary objectives only (6.3.1).

6.4.2. Efficacy

The secondary efficacy parameters include the following assessments:

F. Biomarkers

Biomarkers to be assessed as secondary efficacy endpoints will be determined

- at screening visit;
- before the 1st treatment in all Parts; and
- at end-of-study visit in Parts 2 and 3.

The biomarkers are a part of the physical examination of the eyes and include the following methods:

- blink rate (blinks per minute)
- corneal staining (Oxford grading)
- conjunctival staining (Oxford grading)

Blink rate is further converted to the interblink interval (IBI) and used with TBUT for calculation of the ocular protection index (OPI = TBUT/IBI) (Ousler et al. 2008).

7. DATA MANAGEMENT AND STATISTICAL ANALYSES

Data management and statistics will be carried out by 4Pharma Oy, Turku, Finland.

7.1. Estimation of sample size

Sample sizes in Parts 1 and 2 of this study are based on clinical considerations with no formal sample size calculation. The number of subjects planned to be included in Part 3 is based on expected mean OSDI values shown in Table 3 below. The estimated minimum number of patients completing the study in one treatment group is 23 based on the sample size calculation. Sample size was calculated using nQuery advisor software (version nQuery + nTerim 3.0).

A total number of 64 adult patients (both males and females) will be included in the study as follows:

Part 1. 3 subjects to obtain preliminary information on the acute safety and ocular tolerability of the treatment from all 3 completing subjects (n=3).
Part 2. 9 subjects to obtain preliminary information on the long-term safety, ocular tolerability, and efficacy of the daily treatments from at least 8 completing subjects (n=8).
Part 3. 52 subjects to obtain information on the long-term safety, ocular tolerability, and efficacy of the daily treatments from at least 46 (88%) completing subjects allocated in two randomized treatment groups (n=46).

Table 3. Parameters used in sample size estimation for Part 3

Parameter	OSDI at D1 (baseline)	OSDI at D30 (end of study)	SD
Study treatment group	36	12	14
Control group	36	24	14
Two-sided significance (α)	0.05		
Power (1-β)	0.80		
Total sample size required	46		
Sample size required per group	23		

7.2. General statistical methods

7.2.1. Statistical analysis plan (SAP)

A statistical analysis plan (SAP) including details for the analysis of each study parameter will be written by 4Pharma and finalized before database lock.

7.2.2. Analysis populations

Intent-to-treat (ITT) population. The ITT population includes all randomized subjects who have received the study medication at least once and who have subsequent efficacy measurements available.

Safety population. The safety population will include all randomized subjects who have received the study medication at least once from whom at least one safety measurement is obtained after randomization.

Per-protocol (PP) population. The PP population excludes subjects with significant protocol deviations, including withdrawn subjects. Protocol deviations will be assessed prior to database lock.

For all study Parts, separate analysis populations will be defined.

7.2.3. Statistical hypotheses

The main objective of the study is to evaluate ocular safety and tolerability in 3 different study Parts conducted sequentially. For the study part 3 a sample size calculation was conducted with OSDI as the parameter of interest. The evaluation of the study will be based on the total evidence collected, not only this single parameter in one Part which was used for sample size calculation.

However, for OSDI in Part 3, the following statistical hypothesis will be tested:

H_0: There is no difference between study products in OSDI scores.

H_1: There is a significant difference in OSDI scores in favour of the investigational product.

Statistical analyses will be performed primarily on the ITT population. The PP population will be used as a secondary dataset.

7.2.4. General statistical considerations

Descriptive statistics by treatment group and visit day will be provided to summarize the results. Also changes from baseline will be summarized with descriptive statistics. No imputations will be done for missing observations. If not stated otherwise, a two-sided p-value less than 0.05 will be considered statistically significant.

No interim analyses are planned.

7.2.5. Analysis of study variables

Demographic and baseline characteristics of the subjects will be tabulated with descriptive statistics. The disposition of the subjects (and eyes) will be summarized.

The safety population having received the study treatment and with any follow-up data will be considered evaluable for safety and tolerability for the corresponding Part of the study. AEs will be summarized for each treatment group and study Part by severity, causality, and duration. Ocular AEs will be reported separately. All SAEs will be listed with all relevant information.

In Part 1, the ocular safety and tolerability of trehalose emulsion eye drops will be evaluated as a within-subject comparison to the untreated eye and to baseline. In Part 2, the evaluation will be performed as a within-subject comparison to the control eye and to baseline. In Part 3, the evaluation will be performed between subjects receiving trehalose emulsion eye drops in comparison with subjects receiving control eye drops and to baseline.

Physical examination of the eye parameters will be summarized descriptively by eye in all Parts of the study. The ocular safety and tolerability of the treatments evaluated at the end-of-study visit will be summarized by study day, treatment and eye. In addition, ocular comfort ratings (OSDI) by the subjects will be summarized by study day and treatment (Parts 2 and 3).

The comparisons between treatments in Part 2 will be conducted using standard statistical methodology for paired data (e.g. paired t-test for continuous variables and Wilcoxon signed rank test for variables in ordinal scale). The comparisons between treatments in all ocular measurements (including biomarkers and physical examination of eye parameters) in Part 3, which are the primary basis of evaluation of study objectives, are done with statistical methods appropriate for parallel group design. Analysis of variance technique will be used to evaluate the difference in the change in OSDI between treatment groups. Wilcoxon rank sum test will be used for ordinal scale variables (e.g. staining and redness). Details of all methods and the testing strategy will be provided in the SAP finalized before the database lock and randomization code opening.

Concomitant medications will be listed.

7.3. Data management

Data management is the responsibility of the 4Pharma. Further details are given in a study-specific data management plan.

8. QUALITY CONTROL AND QUALITY ASSURANCE

The principles of good clinical practice (GCP) as expressed in ISO 14155:2011 are followed.

The investigational devices will be manufactured at the Sponsor's manufacturing site under the quality management system conforming to EN ISO 13485:2012 and ISO 13485:2003 + Cor. 1:2009. Real-time stability of both devices for the duration of the clinical study will be confirmed in product validation studies before the initiation of the clinical study.

The standard operating procedures of 4Pharma are followed for data management and statistics.

Competent authorities or the Sponsor may conduct audits in any phase of the study.

Curriculum vitae will be obtained from the Investigator who will sign the protocol.

9. ETHICAL CONSIDERATIONS

This study will follow the relevant regulations and guidance for biomedical research involving human subjects, such as the Declaration of Helsinki, the GCP, EN ISO 14155, and the European and national regulations. Special emphasis will be put on the well-being of the subjects.

The investigational devices deliver doses in a relatively narrow volume range. The doses and dose frequency to be administered in this study have been selected based on existing clinical practice and data on preclinical and clinical efficacy, safety and toxicity, as presented in the IB. No specific safety concerns are expected. Local tolerability and safety will be followed after the treatments, and the duration of repeated dosing is increased during the course of the study by a step-wise approach from Part 1 to Part 3. Most of the ingredients of the investigational device Piiloset Trehalose Emulsion Eye Drops have been used in patients with moderate or severe dry eye in a clinical investigation for [deleted].

The clinical safety and tolerability of the ingredients have further been evaluated in the IB. A new ingredient of the investigational device is sacha inchi seed oil [deleted] which has not previously been reported to have been used by ocular administration in human subjects. There are multiple reports, however, on the safety and tolerability of sacha inchi seed oil by dermal administration (CIR 2011). The composition of sacha inchi seed oil is very similar to [deleted]. The control eye drop is a conventional formulation of hyaluronic acid in [deleted] with well-established safety profile. Both investigational formulations are free of preservative agents and packed in similar eye dropper devices. The eye dropper device designed for preservative-free formulations has an established position on the market [deleted].

Prior to initiation of the study, the study protocol, subject information leaflet, informed consent form, and description of the personal data file will be submitted to the EC. The EC will also be notified of other materials to be given to the subjects, such as a draft sample of the study diary.

The study subject candidate (or his/her legally designated representative, if applicable) will be provided with both verbal and written information on

- the nature, objectives, benefits, implications, risks and inconveniences of the clinical investigation;
- the subject's rights and guarantees regarding his or her protection, in particular his or her right to refuse to participate in and the right to withdraw from the study at any time without any resulting detriment and without having to provide any justification;
- conditions under which the clinical investigation is to be conducted, including the expected duration of the subject's participation in the clinical investigation; and
- procedures and follow-up activities upon discontinuation or termination.

The candidate is encouraged to ask questions on the study. After having been given adequate time to consider the decision to participate, no study procedure will be implemented prior to obtaining a written informed consent signed by the subject at the time of consent. The subject will receive a copy of the signed consent. The Investigator will keep the signed consent forms secured on file for inspection by a regulatory authority at any time.

The subjects will be explained what information of them is collected and of its confidential storage. Description of the Personal Data File is kept in the ITF and made available for inspection. The Investigator will assure that the privacy of the subjects, including their personal identity and all other medical information, will be maintained at all times.

The subjects are urged to report all AEs or any other urgent study-related issues by calling the Investigator or Study Nurse in phone numbers given.

10. DATA HANDLING AND RECORD KEEPING

10.1. Case report form (CRF)

All subject data collected during the study will be recorded on CRFs. Only authorized persons agreed between the Investigator and the Sponsor are allowed to make entries on CRFs. The original CRFs remain the property of the Sponsor.

10.2. Source data

The data appearing only on CRFs will be regarded as source data. Access to the source data revealing the identity of the study subjects is only to study personnel. The generated source data are stored within the ITF at the study centre.

10.3. Deviations

In case the Investigator, the Study Nurse or other authorized person involved in the study observes a protocol deviation, he/she should describe the issue as clearly as possible in a dated and signed memorandum. The Investigator will also sign the memorandum. Deviations concerning a single study subject will be described on the corresponding CRF.

11. STUDY SCHEDULE

[deleted]

12. AMENDMENTS TO STUDY PROTOCOL

Substantial amendments to the approved clinical study protocol (which are likely to have a substantial impact on the safety, health or rights of the subjects or on the robustness or reliability of the clinical data generated) will be implemented only with a favourable written opinion issued by the EC, except when taking appropriate urgent safety measures to protect the subjects against any immediate hazard. The EC and Valvira will be notified of such safety measures and of any substantial amendments.

The EC and Valvira will also be notified when the study has been completed after the last visit of the last subject. A clinical investigation report and a report summary will be prepared by the Sponsor and submitted to Valvira.

Minor changes such as those concerning logistics or administrative issues can be clarified in a memorandum or in a non-substantial amendment, if the change has no effect on the safety of the subjects or on the scientific value of the study. The Investigator will inform the Sponsor of such minor changes.

Amendments to the clinical study protocol are prepared as agreed between the Investigator and the Sponsor.

13. PREMATURE STUDY TERMINATION

The study may be discontinued on the decision of the Investigator after consulting the Sponsor. The decision can be based on the of the following factors:

- Occurrence of AEs previously unknown in respect to their nature, severity, and duration
- Medical or ethical reasons affecting the performance of the study
- Difficulties in the recruitment of subjects
- Significant deviations from the protocol

The Investigator will inform the Sponsor immediately and the Sponsor will inform Valvira and the EC within 15 days if the study is terminated prematurely. The Sponsor reserves the right to prematurely terminate the

study for valid scientific or administrative reasons. The Investigator will take appropriate actions concerning the study subjects in the case of premature termination.

14. FINANCING AND INSURANCE

Financing is agreed in contracts between Oy Finnsusp Ab, Kuopio University Hospital District, 4Pharma Oy, and other relevant parties involved. The Sponsor follows an insurance policy covering damages caused by the investigational devices administered during the course of the study. The insurance statement will be provided in the ITF. Incidents of injury causally related to study procedures but unrelated to the investigational devices will be covered by the patient injury insurance of Kuopio University Hospital District.

15. REGISTERING, REPORTING AND PUBLISHING

The EC and Valvira will be notified about study completion according to relevant laws and regulations. A study report summary will be prepared after the study has been completed (or if prematurely terminated). The report summary will be approved by the Investigator and the Sponsor. The Sponsor remains the exclusive owner of the study data defined in the protocol. The results of the study will be published in an international peer-reviewed journal with the Investigator as one of the authors.

The clinical study and its protocol will be pre-registered in a public study registry (such as at clinicaltrials.gov) in advance.

16. ARCHIVING

All information is handled confidentially and according to the current laws and regulations.

The ITF including screening and source data, signed informed consents, and copies of CRFs will be archived by the study site for possible follow-up inspections and audits by competent authorities according to applicable laws.

Pseudonymized (non-personal coded) information collected during the course of this study will be stored by the Sponsor for use in reporting and in the development of eye care products. This information will not be transferred outside Finland. The information may be stored for at least 5 years and as long as is deemed necessary and the investigative product is being marketed and used.

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