Statistical Analysis Plan

Study Information:

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Study Title:	Randomized, Assessor-Masked, Active-Controlled, Phase 3 Study to Evaluate Efficacy, Safety and Tolerability of 0.08% Polyhexamethylene Biguanide (PHMB) Ophthalmic Solution in Comparison with 0.02% PHMB + 0.1% Propamidine Combination Therapy in Subjects Affected by *Acanthamoeba* keratitis.
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Author:	Christina Ehrenkrona

LINK Medical AS
Jörgen Kocksgatan 4
SE-211 20 Malmö, Sweden

e-mail: Christina.Ehrenkrona@linkmedical.eu

Mobile: +46 706 040 010

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1 Acronyms and Abbreviations used in the Document

Acronyms & Abbreviations	
AE	Adverse event
ANOCVA	Analysis of covariance
ANOVA	Analysis of variance
ATC	Anatomical Therapeutic Chemical (classification system)
BCVA	Best corrected visual acuity
CI	Confidence interval
CDISC	Clinical Data Interchange Standards Consortium
CPMP	Committee for Proprietary Medicinal Products
CRF	Case report form
CRR	Clinical resolution rate
CRR_12	Clinical resolution rate at 12 months after randomization
CS	Clinically significant
DDP	Data Display Plan
dL	Decilitre
EMA	European Agency for the Evaluation of Medicinal Products
EoT	End of treatment
EQ-5D-5L	EuroQol 5 dimensional, 5 levels standardized measure of health status
EU	European Union
FAS	Full analysis set
G	grams
IMP	Investigational medicinal product
ICH	International Conference on Harmonisation
IMP	Investigational medicinal product
IOP	Intralocular pressure
ITT	Intention to treat
L	Litre
LINK	LINK Medical Research AS
LogMAR	Logarithm of the Minimum Angle of Resolution
Mg	Milligrams
MedDRA	Medical Dictionary for Regulatory Activities
Mmol	Millimole
NCS	Not clinically significant
NSAID	Non-steroidal
ODAK	Orphan Drug for *Acanthamoeba* keratitis
PCT	Propamidine combination therapy
PHMB	Polyhexamethylene biguanide
PP	Per protocol
PPAS	Per-protocol analysis set
SAE	Serious adverse event
SAP	Statistical analysis plan
SOC	System organ class
SDTM	Study Data Tabulation Model

Acronyms & Abbreviations	
U	units
VAMS	Visual Acuity Measurement Standard
VFQ25	25-item visual functioning questionnaire
WHO	World Health Organization

2 Introduction

The Orphan Drug for *Acanthamoeba* keratitis (ODAK) is a project aiming to investigate the potential of polyhexamethylene biguanide (PHMB) as a safe and effective drug for the treatment of the rare eye disease *Acanthamoeba* keratitis. This debilitating infectious disease is caused by a commonly occurring protozoan and in the absence of treatment can result in blindness. There are currently no approved drugs to treat this disease.

PHMB has received the orphan drug designation (EU/3/07/498) according to EC regulations 141/2000.

The Statistical Analysis Plan (SAP) is a complementary document to the Clinical Study Protocol and includes a more technical and detailed elaboration of the principal features of the proposed statistical analysis and presentations, and the way in which anticipated analysis problems will be handled.

If the SAP suggests changes to the principal features stated in the protocol, these should also be documented in a protocol amendment. Otherwise, it will suffice to record the changes in the SAP.

3 Study Objectives

3.1 Definitions

Clinical resolution is a clinical definition, resulting from slit lamp examination, with the following findings:

- No corneal inflammation (including subepithelial infiltrates, stromal infiltrates and oedema) that requires treatment, with a healed corneal epithelium and minimal punctate staining (10 dots or less equivalent to Grade 1 on the Oxford Scale).
- No or mild conjunctival inflammation (including bulbar injections, bulbar oedema, tarsal hyperemia): mild conjunctival inflammation is acceptable if related to other concurrent conditions such as blepharitis.
- No limbitis, scleritis or anterior chamber inflammation.
- No relapse within 30 days of discontinuing all topical and systemic therapy given for *Acanthamoeba* keratitis.

For regulatory and verification reasons there is an additional 60-day follow-up to exclude late relapses.

The CRR_12 is the clinical resolution rate at 12 months from randomisation and is defined as the percentage of subjects cured 30 days after discontinuing all study therapies, with 12 months of randomisation. This is the primary outcome measure. Subjects whose disease has not resolved at 12 months are considered treatment failures per protocol.

3.2 Primary Objective

The primary objective of the study is to compare the Clinical Resolution Rate (CRR) at 12 months from randomization (CRR_12) of 0.08% PHMB + placebo (Group 1), with that of 0.02% PHMB + 0.1% propamidine combination therapy (Group 2) estimating the difference in CRR_12 together with the surrounding degree of uncertainty, and to test for therapeutic superiority or non-inferiority of 0.08% PHMB monotherapy.

3.2.1 Primary Variables

The primary efficacy variable is the CRR_12.

The CRR_12 is the clinical resolution rate at 12 months from randomization and is defined as the percentage of subjects cured 30 days after discontinuing all study therapies, within 12 months of randomization.

Criteria for clinical resolution:

A subject will be considered cured if resolution or absence of all the following clinical signs is confirmed by a slit lamp examination:

- No corneal inflammation (including subepithelial infiltrates, stromal infiltrates and oedema) that requires treatment, with a healed corneal epithelium and minimal punctate staining (10 dots or less equivalent to Grade 1 on the Oxford Scale).
- No or mild conjunctival inflammation (including bulbar injection, bulbar oedema, tarsal hyperemia): mild conjunctival inflammation is acceptable if related to other concurrent conditions such as blepharitis.
- No limbitis, scleritis or anterior chamber inflammation. No relapse within 30 days of discontinuing all topical and systemic therapy given for *Acanthamoeba* keratitis.

For regulatory and verification reasons, there is an additional follow-up at Day 90 after last treatment to exclude late relapses. Subjects whose disease has not resolved at 12 months are considered treatment failures per protocol.

3.3 Secondary Objectives

Secondary objectives of this study are to obtain additional safety information on 0.08% PHMB ophthalmic solution.

Two secondary hypotheses have been specified in the protocol:

- That adverse events (AEs), and those relating to toxicity in particular, are less with PHMB 0.08% monotherapy compared to the comparator
- Time-to-cure is shorter in subjects receiving PHMB 0.08% monotherapy compared to the comparator

3.3.1 Secondary Efficacy Variables

The following secondary variables were defined in the protocol:

- Best corrected visual acuity (BCVA), i.e. the eyesight test 20/20 (6/6 in metric units; the Snellen notation)
- Corneal scarring as identified by slit lamp examination
- Ulceration severity as identified by slit lamp examination using a 2-grade scoring procedure, i.e. present or absent
- Anterior chamber inflammation as identified by ophthalmoscopy using a 4-grade scoring procedure
- EQ-5D-5L questionnaire
- VFQ25 (self-administered format) questionnaire version 2000, without optional items

In addition, Time-to-cure will be assessed. Other variables may be analysed in an explorative approach. Some disease aspects will be recorded in fields of type *Any other abnormalities? If Yes, Specify.* Such information will be assessed, converted to *Absent/Present* variables and analysed if sufficient numbers of occurrence are seen. Such findings will be tabulated.

3.3.2 Secondary Safety Variables

The following safety variables were defined in the protocol:

- AEs
- Clinical laboratory tests
- Intraocular pressure (IOP)
- Ophthalmoscopy
- Worsening of the corneal epithelial defect and definable inflammatory signs (development of ring abscess and hypopyon) despite >30 days of treatment with the study drug)
- Rate of subjects with a relapse of infection
- Rate of subjects requiring surgery, including amniotic membrane transplants, superficial keratectomy, application of cyanoacrylate glue, therapeutic penetrating, lamellar keratoplasty, cataract surgery, evisceration, or enucleation
- Rate of subjects requiring non-study therapies, e.g., topical steroids and NSAIDs
- Rate of subject discontinuation from study: to permit alteration of anti-amoebic therapy or for other unrelated specified reasons
- Incidence of secondary complications, such as significant corneal neovascularization, corneal scarring, corneal perforation, scleritis, secondary glaucoma, cataract, retinopathy

3.4 Hypothesis

The primary hypothesis to be tested is that the CRR_12 of subjects treated with 0.08% PHMB monotherapy, is superior, or worse by no more than an acceptable pre-defined 0.20 non-inferiority margin (Δ), compared to the CRR_12 of a 0.20 PHMB + 0.1% propamidine combination therapy, administered according to the Treatment and Follow-up Protocol described in Section 5.3, which is based on a consensus of currently clinical guidelines.

Secondary hypotheses are:

- That adverse events, and those relating to toxicity in particular, are less with PHMB 0.08% monotherapy compared to the comparator.
- That time to a cure is shorter in subjects receiving PHMB 0.08% monotherapy compared to the comparator.

Note: The estimated CRR_12 from the start of treatment is 67% for the conventional (0.02% PHMB + 0.1% propamidine) combination therapy (from the sponsor's observational, case series retrospective study 038/SI20) (63% when assuming a prevalence of late stage diseases in 38% of subjects).

4 Study Design

This is a randomised, assessor-masked, active-controlled, multiple centre, parallel-group Phase 3 study to evaluate the efficacy, safety and tolerability of 0.08% PHMB ophthalmic solution compared to the conventional 0.02% PHMB + 0.1% propamidine combination therapy in male and female subjects affected by *Acanthamoeba* keratitis.

The study is designed as a superiority study with the possibility to test for non-inferiority if the superiority hypothesis is not met, according to the requirements of the guidance from the European Agency for the Evaluation of Medicinal Products (EMA) (CPMP/EWP/482/99).[21] The specific conditions for this outcome are described in section 7.4 of the protocol.

The study consists of an eligibility screening visit, a treatment period including short ambulant visits, and follow-up visits.

Group 1: 0.08% PHMB + placebo

Group 2: 0.02% PHMB + 0.1% propamidine combination therapy (PCT)

The study will be performed in male and female subjects affected by *Acanthamoeba* keratitis, ≥12 years of age, inclusive. The inclusion- and exclusion criteria are presented in Chapter 4 of the protocol.

5 Study Population

The study will be performed in subjects affected by *Acanthamoeba* keratitis.

Screening will be performed on the day of the first drug administration (Day 0), or up to two days before study drug administration. The specific inclusion and exclusion criteria can be seen in sections 4.1 and 4.2 of the protocol.

5.1 Sample Size

A total of 130 subjects affected by *Acanthamoeba* keratitis will be assigned to one of the above two treatment groups in a ratio of 1:1.

The protocol states, in section 7.6:

"From the results of the sponsor's observational, case series retrospective study 038/SI, a CRR_12 of 67% for the conventional combination therapy of PHMB 0.02% and propamidine 0.1% is expected. This figure is in the range of cure rates described in the literature. If the true difference in CRR_12 is 0.20 (Δ) (or more) in favour of PHMB monotherapy (0.08% PHMB), a total sample size of 116 subjects (allowing for 10% loss to follow-up) should give the study at least 80% power to detect superiority, with 2-sided alpha=0.10 (or equivalently 1-sided alpha=0.05).

Assuming a prevalence of late stage disease, and worse outcomes, in 38% of subjects, the expected CRR_12 in the control group will be reduced from 67% to 63%. To account for the inclusion of this group, the sample size has been adjusted to 130 subjects (allowing for 10% loss to follow-up, i.e. 116 evaluable subjects).

5.2 Randomisation

After obtaining oral and written informed consent, subjects meeting the eligibility criteria were randomized to one of the treatment groups in a 1:1 ratio. The randomization schedule was generated using a computer program and verified for accuracy using strict quality control procedures. Eligible patients received a masked treatment assignment with a unique randomization code based on the randomization list. The assigned randomization code was captured in the electronic Case Report Form (eCRF). This randomization code was designed as not to disclose any treatment assignment, see section 5.1 of the protocol.

Until the moment of unmasking, the treatment assignments linked to the codes should only be accessible for the individuals appointed by the Sponsor as Qualified Persons responsible for Pharmacovigilance. Emergency Unmasking should only be done if knowledge of treatment assignment was considered relevant for medical care of the patient. The randomization code will be broken when all subjects have finished the study at the formal request from the study statistician after the Clean file report has been signed, any early unblinding has been accounted for and the database has been locked.

6 Assessments

6.1 Primary efficacy endpoint criteria for assessing clinical resolution

A subject will be considered cured if resolution of all the following clinical signs are observed, resulting from a slit lamp examination:

- No corneal inflammation (including subepithelial infiltrates, stromal infiltrates and oedema) that requires treatment, with a healed corneal epithelium and minimal punctate staining (10 dots or less equivalent to Grade 1 on the Oxford Scale).
- No or mild conjunctival inflammation (including bulbar injection, bulbar oedema, tarsal hyperemia): mild conjunctival inflammation is acceptable if related to other concurrent conditions such as blepharitis.
- No limbitis, scleritis or anterior chamber inflammation.
- No relapse within 30 days of discontinuing all topical and systemic therapy given for *Acanthamoeba* keratitis.

For regulatory and verification reasons, there is an additional 60-day follow-up to exclude late relapses.

Relapse of infection is identified by a positive culture, unfortunately very insensitive due to the persistence of deep organisms, supported by an increase in cysts on confocal (also insensitive in severe disease) or using clinical criteria: development of more severe corneal inflammation, melting, ulceration hypopyon, development of ring abscess necessitating another intensive course of therapy.

The primary hypothesis to be tested is that the CRR_12 of subjects treated with 0.08% PHMB monotherapy, is superior, or worse by no more than an acceptable pre-defined 0.20 non-inferiority margin (Δ), compared to the CRR_12 of 0.02% PHMB + 0.1% propamidine combination therapy, administered according to the Treatment and Follow-up Protocol presented on page 16 of the Protocol.

6.2 Secondary efficacy endpoints assessment

6.2.1 Assessment of BCVA

BCVA is being determined using pinhole with or without spectacles, soft contact lenses or rigid contact lenses. Detailed information about the procedure is provided in the Study Operation Manual. International Council of Ophthalmology approved a Visual Acuity Measurement Standard (VAMS) in 1984 (3). In Table II in the VAMS different types of notations of visual acuity are presented, one being the Snellen notation, such as 20/16 when measured in feet or 6/4.8 when measured in metres. Other notations are the decimal notation, 20 divided by 16 (6 divided by 4.8) which equals 1.25, the visual angle (the inverse of the decimal notation, 0.80) or the logarithm (base 10) of the visual angle, referred to as LogMAR.

In addition, refraction will be assessed, together with any potential relationship between BCVA and refraction.

6.2.2 Assessment of corneal scarring

Corneal scarring is being assessed as *Present* or *Absent*, using the slit lamp biomicroscopy performed by the examining ophthalmologist. Detailed information about the procedure is provided in the Study Operation Manual.

6.2.3 Assessment of ulceration severity

Ulceration severity is being assessed as *Present* or *Absent* by the examining ophthalmologist using the slit lamp biomicroscope.

6.2.4 Pupil test

Pupil test (swinging light test) is reported as Normal; Abnormal, NCS; Abnormal CS. The specific abnormalities will be listed.

6.2.5 Assessment of anterior chamber inflammation

Anterior chamber inflammation is being assessed using a 3-grade scoring procedure using the slit lamp biomicroscope: Abnormal Clinically significant (CS), Abnormal Not CS (NCS), and Normal. Clinically significant events are defined as “any variation, symptoms, or testing that has medical relevance according to the investigator and may result in an alteration in in medical care".

6.2.6 EQ-5D-5L and VFQ25 assessments

EQ-5D-5L and VFQ5 are two instruments for subject-reported outcomes; the former addressing the overall health status of the subjects and the latter their visual function status. More detailed information can be seen in appendices 2 and 3 of the protocol.

6.3 Secondary safety endpoints assessment

6.3.1 AEs

Recording of AEs will commence with signing of the ICF. AEs will be captured in the CRF. AEs will be followed up by the investigator as specified in section 8 of the protocol, AE Reporting. Standard CDISC STDM annotation is being used for AE data.

6.3.2 Clinical laboratory tests assessment

Clinical laboratory results (haematology, biochemistry and urinalysis) were conducted at the Screening or Baseline visit and at the End-of-study visit. These are annotated using standard SDTM names. Clinical laboratory tests will be presented with summary statistics. In addition, the tests will be analysed with ANCOVA (baseline value of respective laboratory test and treatment group will be included in the model). All results will be regarded as descriptive only. The p-values will be used to flag safety and tolerability variables worthy of further attention.

7 Methods of Analysis

7.1 General

All statistical analyses will be performed in accordance with the ICH E9 guideline for Statistical Principles for Clinical Trials (1), using SAS® (Version 9.4 or higher, SAS Institute Inc., Cary, NC, USA).

7.1.1 Presentation of Results

All results will be presented for each visit by treatment group and in total, unless stated otherwise. It should be clearly stated which unit applies to each presented variable.

Continuous data will be summarised using descriptive statistics, and the following parameters will be reported:

- number of subjects with evaluable observations and missing observations
- arithmetic mean and change from baseline with standard deviations

- median
- first and third quartiles
- minimum and maximum

Categorical data will be presented using absolute frequency and percentage. When the absolute frequency is zero, the percentage will not be presented. Unless stated otherwise, the denominator for percentage calculations will be the total number of subjects in the applicable analysis set, including subjects with missing data. For variables with missing values, the number and percentage of subjects with missing values will be presented.

Significance tests will be two-sided and performed at the 5% significance level, unless stated otherwise. When reporting the results of significance tests, p-values will be presented.

All confidence intervals (CIs) presented will be two-sided with a nominal confidence level of 95%, unless stated otherwise.

Data will be presented using an appropriate number of decimal places, to ensure that undue precision is not implied (*e.g.* the number of decimals should not exceed the accuracy of the measuring instrument). Raw data will be presented with the same number of decimals as collected, and derived data with an appropriate number of decimals based on general practice, mathematical rationale or scientific rationale.

Minimum and maximum values will be presented with the same number of decimals as the analysed variable and the other descriptive statistics will be presented with one decimal more. Percentages and proportions will be presented with one decimal. Odds ratios and hazard ratios will be presented with 3 decimals, and small and large values will be presented as '<0.001' and '>999.999' respectively. CI bounds will be presented with the same number of decimals as the corresponding point estimate, and p-values will be presented with 4 decimals or as '<.0001'.

Mock tables and graphs are presented in the Data Display Plan (DDP), which is a supplementary document to this analysis plan. Individual subject data listings will be presented according to the ICH E3 guideline for Structure and Content of Clinical Study Reports (2), unless stated otherwise.

7.1.2 Baseline

Unless stated otherwise, the baseline value for a parameter is defined as the last non-missing value before the first dose of the investigational medicinal product (IMP).

7.1.3 Analysis Relative Day

The analysis relative day for an assessment/value is defined as the time in days from the date of randomization to the date of the assessment. The date of randomization is considered as day 1, and earlier dates will correspond to a negative day. In the Schedules of assessments table on page 22 and 23 of the protocol, the day of randomisation, which also encompass some baseline measurements and drug dispensing, is called Day 0. As CDISC does not use 0 for day numbering, Day 1 will be used for the day of randomisation. Hence, the day numbering after the baseline day in the Schedules table will be increased by one.

7.1.4 Analysis Visit

An analysis visit is defined as a categorical variable used to classify values within an analysis variable into temporal or conceptual groups used for analyses.

The visits as defined in the case report form, CRF, will be used as analysis visits. The Schedule of Assessments table provide acceptable deviations from the specified study day at which study visits should occur. These are ± 2 days up to study day 22, called study day 21 in the Schedules table, ref. section 7.1.3 above, ± 3 days from study day 31 to 30 days after end of treatment (EoT), and ± 5 days at the 90 day after end of treatment visit. A review of assessment times will be conducted before the clean file meeting. Necessary revisions will be documented in the Clean file report. Results tables will present statistics by visit number. Where deemed necessary, longitudinal analyses will use the observed durations from baseline. The mean duration in days between visits will be presented.

Study day windows

Study visit day	Target study day of visit	Window of study day visit
0	0	± 0 days
7	7	± 2 days
14	14	± 2 days
21	21	± 2 days
30	30	± 3 days
60	60	± 3 days
30 days after previous visit§ until resolution	30 after previous visit	± 5 days
30 days after EoT	30 days after EoT	± 3 days
90 days after EoT	90 days after EoT	± 5 days

§ The protocol states "and every 30 ± 5 days until resolution". This is interpreted as 30 days after the previous visit, and not a multiplicity of 30 days based on a fixed setup from baseline, i.e. not 31+60+n*30.

In general, data from unscheduled visits will be presented in data listings only and not included in analysis or summary tables. An exception to this is data used to confirm eligibility in association with screening or randomisation where the last assessment will be considered in summaries of screening data.

7.1.5 Handling of Missing Data

Sensitivity analyses performed for a single variable are described in detail in the corresponding section below.

Data listings will include the observed values. For derived variables, values based on imputed data will be presented in listings.

7.1.6 Interim Analyses

No interim analysis is planned.

7.1.7 Multiplicity

Three hypotheses are specified in the Protocol, one primary and two secondary hypotheses. Time-to-resolution using the visit day at which resolution was first seen, will be considered statistically significant if the sum of the p-value for the primary variable and the p-value from the time-to-resolution analysis is less than the specified overall significant level. The use of a non-inferiority alternative if the difference alternative should not be obtained, does not affect the overall p-value level of the primary hypothesis.

Hypotheses tested for other secondary variables will all be analysed using significance elves of 0.05.

7.1.8 Subgroups

No subgroup analyses will be performed.

7.2 Analysis Sets

The decision on the classification of subjects to each analysis set will be taken at the clean file meeting, prior to breaking the blind, and documented in the clean file report together with the reasons for excluding subjects from analysis sets. Subjects who have not attended any visits where disease status is assessed, i.e. those who did not reach the day 30 visit, the first visit where disease status is recorded, will be classified as dropouts from the efficacy analysis sets. Any 30dayFU visit that is under the window, i.e. visit occurred too soon, will not be removed from the analysis population as long as the 90DayFU visit also proved that the patient was cured.

7.2.1 Full Analysis Set (FAS)

All subjects who have been randomized and has a confirmed diagnosis of Acanthamoeba keratitis and for whom the primary efficacy variable has been assessed will be included in the FAS. The FAS will be used in all efficacy analyses. The last disease status assessment will be used for disease status assessment.

Analysis using the FAS will be based on the planned treatment (i.e. subjects will be analysed 'as randomised').

7.2.2 Per-Protocol Analysis Set (PPAS)

The per-protocol analysis set is defined as the subset of subjects in the FAS for whom no major protocol deviation judged as having an impact on the primary efficacy analysis was reported or identified. The last disease status assessment within 12 months of randomisation will be used for disease status assessment. For subjects with disease resolution at that last visit, the 30-day and 90-day follow-up assessments should confirm disease resolution. Otherwise, the disease will be regarded as not having been resolved.

The decision as to which protocol deviations should be considered as reason for exclusion from the PPAS should be made at the clean file meeting and documented in the clean file report.

Analysis on the PPAS will be based on the actual treatment (i.e. subjects will be analysed 'as treated'). The primary efficacy variable, CRR_12, will also be evaluated using the PPAS.

7.2.3 Safety Analysis Set

All subjects who have received at least one dose of study medication will be included in the Safety Analysis Set. This set will be used in all analyses of safety data.

Analysis on the safety analysis set will be based on the actual treatment (i.e. subjects will be analysed 'as treated').

7.3 Disposition of Subjects

The following will be presented:

- Number of screened subjects, in total, if available.
- Number of screening failures, in total, if available.
- Number of randomised subjects, by treatment group and in total.

Based on the number of randomised subjects, the following will also be presented, by treatment group and in total:

- Number and percentage of subjects who did not meet randomisation inclusion/exclusion criteria
- Number and percentage of subjects who did not receive any dose of IMP.
- Number and percentage of subjects who received at least one dose of IMP.
- Number and percentage of subjects who completed the required treatment.
- Number and percentage of subjects who withdrew prematurely from the study, i.e. withdrew before completing the follow-up visits.
- Number and percentage of subjects in each of the analysis sets.

In addition, a frequency table on the primary reason for premature withdrawal from the study will be presented by treatment group and in total. Percentages for this table will be based on the number of prematurely withdrawn subjects.

The number of subjects attending each study visit will also be summarised.

7.4 Protocol Deviations

Protocol deviations will be presented in a data listing.

The number and percentage of randomized subjects with at least one major protocol deviation leading to exclusion from an analysis set will be presented.

Major deviations are those deviations that could affect the scientific integrity of the study:

- Did not comply with the allocated treatment
- Did not attend the two follow-up visits

7.5 Demographics and Baseline Characteristics

Summary statistics and frequencies on demographic data (age and sex) will be presented for all analysis sets.

In addition, the summary of Best sphere (dioptres) will be summarised for the following categories:

Hypermetropes (Refraction greater than 0)

Emmetropes (Refraction equal to 0)

Myopes (Refraction less than 0)

7.6 Medical History and Concurrent Diseases

Medical history and concurrent diseases will be coded according to the latest available version of Medical Dictionary for Regulatory Activities (MedDRA).

For each system organ class and preferred term, the number and percentage of subjects with at least one condition in that system organ class or preferred term will be presented. Medical history and concurrent diseases will be presented in separate tables, based on the safety analysis set.

Medical history is defined as events stopped prior to study inclusion. Concurrent diseases are defined as ongoing events and events stopped on or after baseline. If the start and/or stop date is partially unknown, the following imputation rules will be used for the purpose of classifying the events:

Table: Imputation of missing parts of dates

	Imputed start date	Imputed end date
Unknown year	Missing	Missing
Unknown month	1 January	31 December
Unknown day	First of month	Last of month

If it is not possible to classify the condition based on the reported and/or imputed start and end dates, it will be considered as concurrent. In data listings, the dates will be presented as reported.

7.7 Prior and Concomitant Medication

Medications will be coded according to the World Health Organization (WHO) anatomical therapeutic chemical (ATC) classification system and summarized by therapeutic subgroup (ATC level 2) and preferred name.

For each therapeutic subgroup and preferred name, the number and percentage of subjects who used at least one medication of that therapeutic subgroup or preferred name will be presented. Prior and concomitant medications will be summarized in separate tables, based on the safety analysis set.

If a reported medication cannot be coded with a preferred name, the lowest available higher-level dictionary term will be used instead in the summary tables. If a medication cannot be coded on a lower level than the therapeutic subgroup or the anatomical main group (ATC level 1), that medication will be presented as ‘Not codable’ under that therapeutic subgroup/anatomical main group.

Prior medication is defined as medication stopped prior to baseline. Concomitant medication is defined as ongoing medication or medication stopped on or after baseline. If the start and/or stop date is partially unknown, the following imputation rules will be used for the purpose of classifying the medication:

	Imputed start date	**Imputed end date**
Unknown year	Missing	Missing
Unknown month	1 January	31 December
Unknown day	First of month	Last of month

If it is not possible to classify a medication based on the reported and/or imputed start and end dates, it will be considered as concomitant. In data listings, the dates will be presented as reported.

7.8 Compliance

Subjects who miss up to 1 full day of treatment within the first 5 days after starting treatment or up to 2 full days of treatment after the first 5 days of treatment should not be discontinued but will be classified as non-compliant. Subjects will be discontinued from the study if in the opinion of the investigator drug compliance is insufficient.

The rate of compliance with the IMP will be summarised for the FAS. The reasons will be listed.

7.9 Efficacy Evaluation

All efficacy evaluations will be based on the FAS. The PPAS will be used for supportive sensitivity analysis of the primary efficacy variable.

7.9.1 Primary Efficacy Variable

The main endpoint disease resolution 12 months after baseline will be assessed at each visit up to study day 365.

Clinical resolution 12 months after baseline for a patient is defined as:

The percentage of subjects cured 30 days after discontinuing all study therapies, within 12 months of randomization. A clinical resolution classification requires a *Yes* response to the eCRF variable CRYN question: "Was clinical resolution obtained?" at one of the study visits from day 1 up to study day 365 in combination with a response *No* to the eCRF variable RELAPYN question "Did the subject experience relapse since the last visit?" at the pursuing visits, up to the last recording of this information. There should be no relapses after (the last) clinical resolution classification. Discontinued subjects will be regarded as not cured.

For patients defined as fulfilling disease resolution within 12 months, the date of the visit at which annotated CRYN is first answered with a *Yes* will be used as the date of resolution. The time-to-cure secondary variable will be the number of days from randomisation to the visit where resolution was recorded.

Since it is also possible to test for non-inferiority if the superiority hypothesis is not met, additional conditions are specified (see list below) to meet the strict requirements of a non-inferiority study, making it feasible to test for non-inferiority using the results from the superiority analysis, without a further separate analysis. The 90% CI for the difference between the treatments obtained from the superiority analysis gives the necessary information. Position of the lower end of the CI relative to a pre-defined agreed 0.20 non-inferiority margin (Δ) provides the key information for making decisions (conclusions) about non-inferiority.

PHMB has received the orphan drug designation. The use of 90% CI for the difference between the two treatments is to provide a 95% lower CI limit for non-inferiority.

The additional conditions specified to make testing for non-inferiority feasible are the following:

- Pre-defined non-inferiority margin (Δ=0.20) based on both clinical and statistical considerations, i.e., chosen on the basis of external information and not chosen to fit the data.
- Analysis of the FAS and the PPAS, giving similar results with respect to p-values and CIs for the efficacy measure at issue.
- Study design and implementation directed at minimizing deviation from protocol (a particularly important source of bias in non-inferiority studies), e.g. special care to minimize violations of: entry criteria; non-compliance; withdrawals; loss; missing data; etc. This should help ensure the second bullet point.
- Evidence that the standard treatment is showing its usual (expected) level of efficacy.

These specifications are in accord with the recommendations by the Committee for Proprietary Medicinal Products, in document *Points to consider on switching between superiority and non-inferiority* (CPMP) 482/99. The recommendation regarding similar results with respect to p-values and CIs is founded on that the FAS "is generally not conservative and its role should be considered very carefully" in non-inferiority trials.

Null hypothesis difference:

- No difference between test treatment and reference treatment

Non-inferiority clinical studies aim to demonstrate that the test treatment is no worse than the reference by more than a pre-specified small amount.

Null hypothesis non-inferiority:

- Test treatment is inferior to the reference by Δ or more

CRR_12 will be analysed using a general linear model (GLM) approach with treatment as factor to estimate the difference between success rates for the treatments, and test for difference between treatments. This will be the primary test of efficacy. The new treatment will be declared as non-inferior to the 0.02% PHMB + 0.1% propamidine combination therapy, if the lower limit of the 95% CI satisfies the requirement of a non-inferiority margin of Δ=0.20. The effects of covariate variables on the success rate will also be assessed using the GLM model with treatment, age and sex as explanatory variables. Further explorative analyses will be conducted by adding other, selected variables such as steroid use before treatment start, to the GLM model.

7.9.2 Justification for choice of the non-inferiority margin

On statistical grounds:

Our pre-defined non-inferiority margin of Δ=0.20 is based on previous studies (historical data), i.e., the study referred to in the protocol showing 1/20 cured without treatment being the only data available that can be used to estimate the cure with placebo, and the results of the reference (standard) treatment from the SIFI / Moorfields observational studies, adjusted for inclusion of advanced disease cases in the study. The table below demonstrates the statistical grounds for the choice of Δ. The chosen Δ of 0.20 satisfies the condition that "the test treatment is expected to retain at least 50% of the standard treatment effect over placebo, in order to be considered as noninferior".

Table 1: Selection of Non-Inferiority Margin (Δ) based on statistical issues

		Proportion
A	Cured with no treatment: Historic Data	0.05
B	Cured with Standard Treatment: SIFI Study Data	0.67
Ba	B adjusted for inclusion of stage-3 cases (63/100)	0.63
Ba (lower)	Lower 95% CI bound for Ba (Binomial exact)	0.53
M1	**Ba** (lower) – **A**: the standard treatment effect over placebo	0.48
Δ	Non-inferiority margin	0.20
Proportion of M1 retained by the Test treatment; (M1 – Δ)/M1		**58%**
The Test Treatment retains well over 50% or the Standard treatment effect over placebo		

On clinical grounds:

The Sponsor proposes as clinically acceptable a non-inferiority margin of 0.20: this is within the accepted level for a study of this kind in view of the following:

- A poor response to therapy is common in this disease. Disease progression is slow in this disease (over weeks and months) and clinicians have to assess the effect of treatments every 1-2 weeks, in cases not progressing well, and modify therapies to optimize outcomes. Because of the disease chronicity the use of an ineffective treatment for these periods results in a delayed response, but no serious short term morbidity. The study results may indicate non-inferiority, when at worst the true difference in proportion 'cured' (Combined - PHMB alone) is 0.20. The most likely impact of such a finding on clinical practice will be to encourage the use of PHMB monotherapy as a first line treatment.
- However, if PHMB monotherapy fails in clinical practice during the early stages of treatment, which is to be expected in some subjects, the clinician is unlikely to abandon the good clinical practice of monitoring the disease progress closely and adding other antiamoebics to the therapy when necessary.
- At worst, the impact of the study finding might result in a little delay (matter of days) in starting a combined treatment in some patients, whilst giving a chance for the biguanide mono-therapy to act.

This scenario is unlikely to result in blindness or serious morbidity. It may cause some delay in the clinical resolution.

- By contrast, the possibility that the true difference may be much smaller than 0.2, or even in favour of PHMB monotherapy, will have important beneficial consequences for patients and ophthalmic services.

7.9.3 Secondary Efficacy Variables[1]

Time-to-cure comparison between the treatment groups is specified as efficacy hypothesis and will be subjected to formal statistical testing. Other analyses of secondary efficacy variables will be done on a hypothesis-generating basis, i.e. no p-value adjustments will be conducted, and conclusions regarding the analysis results will be commented on such a basis.

Time-to-cure

The aim of the hypothesis test is to show that the time is less among the subjects receiving PHBM 0.08% monotherapy compared to the comparator. Patients will either be classified as *Cured* at some point of time while in the study or leave the study without being classified as *Cured*. As a secondary efficacy variable, time-to-cure (clinical resolution) will be analysed. The Cox Proportional Hazards regression (subject to validity of the 'proportional hazards' assumption) and Kaplan-Meier survival plots. If the proportional hazard assumption is not fulfilled a logrank test will be performed. Not all patients may have information regarding when they were cured, e.g. due to death before being cured, or completed the study without being cured. Both the Cox Proportional Hazards regression and the Kaplan-Meier plot uses the censoring timepoint in their calculations. The censoring timepoint will be defined based on the timepoint of the last observation when cure status was assessed. The null hypothesis is that the two treatment arms have equal hazard functions. Relevant data will be presented at the Clean file meeting for discussion and acceptance.

BCVA

The VAMS states that: “Depending on the problem, this notation can be most useful when analysing or graphically plotting visual acuity scores because equal linear steps on the LogMAR scale represent equal ratios in the standard size sequence.” Hence, BCVA will be presented and analysed using LogMAR values, where

$$LogMAR = \log\left(\frac{ORRES_{BCA2}}{ORRES_{BCA1}}\right),$$

where ORRES_BCA1 and ORRES_BCA2 are the annotated values from the BCVA test. These values are positive number with up to one decimal value. ORRES_BCA1 values recorded in the eCRF, the distance from the subject to the chart, varies at least from 0.6 to 10 metres. Even if different scales are used (Decimal, US, 6m), the LogMAR is not affected since the ratio will be the same, therefore no converting to unified units is needed[2]. BCV A, as a secondary efficacy variable, will be analysed. ANCOVA will be used with treatment and the Baseline value of LogMAR as predictors. Refraction at Baseline may also be used as a predictor.

Drug compliance

Drug compliance is recorded as *Yes* or *No* answer to the question “Was the study drug administration compliant with the protocol?”, annotated as PROTCOMYN. Drug compliance will be analysed with a logistic regression model and model-based estimates of the difference between treatments, odds ratio with corresponding CIs and p-values will be provided. Covariate variables such as treatment, age and sex will be included. Any available information regarding drug use will also be presented, such as number of vials returned.

EQ-5D-5L[3] & EQ VAS, at all visits

EQ-5D-5L consists of 5 dimensions, *Mobility*, *Self-care*, *Usual activities*, *Pain/discomfort*, and *Anxiety/depression*, each answered with one of five different answers:

Mobility:

I have No problems/Slight problems/Moderate problems/Severe problems in walking about/I am unable to walk about

[1] For safety variables, see Section 7.10.2

[2] RR.2 Reference Tables For Equivalent Visual Acuity Notations

[3] One subject, aged 15 years, used the EQ-5D-Y version. At site 31, Silesia, all subjects at one or more visits used the EQ-5D-3L version

Self-care:

I have No problems/Slight problems/Moderate problems/Severe problems in washing or dressing myself/I am unable to wash or dress myself

Usual activities (e.g. work, study, housework, family or leisure activities):

I have No problems/Slight problems/Moderate problems/Severe problems doing my usual activities/I am unable to do my usual activities

Pain/discomfort:

I have No pain or discomfort/Slight pain or discomfort /Moderate pain or discomfort /Severe pain or discomfort/I have extreme pain or discomfort

Anxiety/depression:

I am not/slightly/moderately/severely/extremely anxious or depressed.

The EQ-5D will be summarised for each dimension. A table presenting the number of subjects reporting a problem, i.e. a score higher than 1, will also be presented. A repeated measures ANCOVA for each dimension will be conducted using treatment and the baseline value as predictors.

The EQ VAS score, annotated as ORRES_EQ5D0206, is being recorded at all visits, including Baseline. Integer values range from 0 to 100. A repeated measures ANCOVA will be used to compare potential differences in trends over the treatment period using treatment and the Baseline VAS value as predictors.

Slit-Lamp Examination, at all visits

Below can be seen the different efficacy variables based on the Slit lamp examination. These will be presented in tables, as can be seen in the Data Display Plan (DDP). Some of these variables will only be recorded in *Specify* types of eCRF fields and will be converted to a *Yes/No* variable before tabulation and possible analysis.

Corneal scarring

Corneal scarring is being assessed as *Present* or *Absent*, using the slit lamp biomicroscopy, and is annotated as SLECOSCAR. This is being performed by the examining ophthalmologist, using a slit lamp biomicroscope. Detailed information about the procedure is provided in the Study Operation Manual. As a stated secondary variable, corneal scarring at EoT will be analysed with logistic regression using treatment group and corneal scarring at baseline as predictors.

Ulceration severity

Ulceration severity is being assessed as *Present* or *Absent* by the examining ophthalmologist using the slit lamp biomicroscope and is annotated as SLECOUL. As a stated secondary variable, ulceration severity will be analysed with logistic regression using treatment group and ulceration severity at Baseline as predictors.

Conjunctiva

Erythema grade SLECER (using grades 0, 1, 2, 3)

Oedema grade SLECED (using grades 0, 1, 2, 3, 4)

Discharge SLECOND (Present, Absent)

Papillae SLECONP (Present, Absent)

Follicles SLECONF (Present, Absent)

Limbitis

Scleritis

Corneal Abnormalities[4]

Dendritiform epithelial lesions

Perineural infiltrates

Corneal epithelial defects (punctate erosions),

Corneal epithelial ulcer (confluent erosions)

Corneal epithelial defects SLECOED (Present, Absent)

Corneal ulceration SLECOUL (Present, Absent)

Corneal epithelial opacity SLECOEO (Present, Absent)

Corneal stromal opacity SLECOSO (Present, Absent)

[4] Some of these may appear an *Any other abnormality* and will be taken from the *Specify* variable

Corneal scarring SLECOSCAR (Present, Absent)

Corneal ring abscess

Any other abnormalities SLECOTHSP (Yes, No), Yes-> Specify

Lens

Lens SLELEN (Normal, Abnormal, NCS, Abnormal, CS)

Anterior chamber inflammation

Anterior chamber inflammation is being assessed using a 4-grade scoring procedure. The annotation is SLEACI with values 0, 1, 2 and 3, signifying *None* (no Tyndall effect), *Mild* (Tyndall effect barely discernible), *Moderate* (Tyndall beam in the anterior chamber is moderately intense), and *Severe* (Tyndall beam in the anterior chamber is severely intense). As a secondary efficacy variable, the anterior chamber inflammation 4-grade scores will be analysed using an ordinal logistic regression approach with treatment and the Baseline anterior chamber inflammation as predictors.

Pupil test, at all visits

Result: PUPTORRES (Normal/Abnormal, NCS/Abnormal, CS)

VFQ-25, at all visits

The Visual Functioning Questionnaire consists of 25 questions, two with one or three sub-questions designed to elucidate the answer to the main question, see Tables 2and 3 below.

Table2: Scoring Key for VFQ-25

Item numbers	Change original response category[a]	To recoded value of:
1, 3, 4, 15c[b]	1 2 3 4 5	100 75 50 25 0
2	1 2 3 4 5 6	100 80 60 40 20 0
5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 16a	1 2 3 4 5 6	100 75 50 25 0 *
17, 18, 19, 20, 21, 22, 23, 24, 25	1 2 3 4 5	0 35 50 75 100

(a) Pre-coded response choices as printed in the questionnaire

(b) Item 15c has four-response levels, but is expanded to a five-levels using item 15b

Note: If 15b=1, then 15c should be recoded to “0”.
If 15b=2, then 15c should be recoded to missing.
If 15b=3, then 15c should be recoded to missing.

* Response choice “6” indicates that the person does not perform the activity because non-vision related problems. If this choice is selected, the item is coded as “missing”.

Table 3: Averaging of items to generate VFQ-25 sub-scales

Scale	Number of items	Items to be averages (after recoding per Table 2)	Annotated variable names, prefix=ORRES_VFQ2
General health	1	1	01
General vision	1	2	02
Ocular vision	2	4, 19	04, 19
Near activities	3	5, 6, 7	05, 06, 07
Distance activities	3	8, 9, 14	08, 09, 14
Vision specific:			
Social functioning	2	11, 13	11, 13
Mental health	4	3, 21, 22, 25	03, 21, 22, 25
Role difficulties	2	17, 18	17, 18
Dependency	3	20, 23, 24	20, 23, 24
Driving	3	15c, 16, 16a	15C, 16, 16A
Colour vision	1	12	12
Peripheral vision	1	10	10

Averaging the means for each scale for each patient, excluding the General health score, provides a composite score for each patient, which can then be used to calculate the composite score for each treatment group.

The mean for each sub-scale per patient is calculated as;

$$Mean = \frac{(Sum\ of\ score\ for\ each\ item\ with\ non-missing\ answer)}{(Total\ number\ of\ items\ with\ non-missing\ answers)}$$

The Visual Functioning Questionnaire consists of 25 questions, two with one or three sub-questions designed to elucidate the answer to the main question. The main questions are annotated as ORRES_VFQ201 to ORRES_VFQ225. The sub-questions are annotated as ORRES_VFQ215A to ORRES_VFQ215C and ORRES_VFQ216A. The values are integer numbers from 1 to 2, 3, 4, 5 or 6 depending on the number of response categories,

7.10 Safety Evaluation

All evaluations of safety data will be performed using the safety analysis set. Some information may only be found in text fields of the eCRF. Evaluations incorporating information not available from the eCRF will thus not be possible.

7.10.1 Extent of Exposure

The protocol specifies the following dosing regimen. On Day 0, the first study drug application will be at the clinical research centre. Thereafter, study drug will be self-administered at home. When the subject is at the clinical research centre for assessments, subjects will apply the study drug themselves at the clinical research centre. On Days 0 to 5, subjects will apply study drug every hour daytime only (1 drop of each ophthalmic solution in the affected eye). On Days 6 to 12, (1 week), subjects will apply study drug every 2 hours daytime only. On Days 13 to 19, (1 week), subjects will apply study drug every 3 hours daytime only. On Day 20 until resolution, subjects will apply study drug 4 times a day at daytime only. Subjects will be treated for the maximum of 1 year after randomization.

Summary statistics on the duration of exposure will be presented.

7.10.2 Adverse Events

AEs data will be captured and encoded by system organ class (SOC) and the lowest level term (Medical Dictionary for Regulatory Activities, current version). A listing of AEs will be created. This listing, at minimum, will contain a description of AEs as to seriousness, severity, onset date and end date, duration, action taken (if any), outcome and likelihood of drug causation ("relation"). A frequency table will be compiled for AEs by treatment group, SOC, and lowest level term. Frequency tables will be compiled showing the number of subjects per treatment group affected by one or more (related) AEs, including percentages, the total number of AEs per treatment group and the average number of AEs per subject exposed to the respective treatment for the following:

- AEs.
- Serious AEs.
- AEs leading to withdrawal of the IMP.
- Fatal AEs.
- AEs, broken down by severity.
- AEs, broken down by causality assessment.

There will also be tables on the most frequently reported AEs, on system organ class level and on preferred term level. The decision on the frequency cut-off for these tables will be taken during the analysis of the AEs data in consultation with the author of the clinical study report and could be influenced by factors such as the overall number of AEs, study design, and the nature of the indication. The frequency cut-off should be mentioned in a table note.

The second secondary hypothesis, as specified in the protocol, is that AEs, and those relating to toxicity in particular, are less with PHMB 0.08% monotherapy compared to the comparator. This translates into:

> Hypothesis: Rate of AEs is equal in the PHMB 0.08% monotherapy is equal to that of the comparator. In particular:
>
> Hypothesis: Rate of toxicity-related AEs is equal in the PHMB 0.08% monotherapy is equal to that of the comparator

The null hypothesis is that rate of occurrence of AEs and toxicity-related AEs are equal for the two treatment arms.

If these are rejected, the conclusion is that the rates are different, and less with PHMB 0.08% monotherapy than for the comparator if the latter rate is higher. The AETOXGR will be used to assess toxicity rates.

These will be tested using ordinal logistic regression model and model-based estimates of the difference between treatments. Odds ratio with corresponding CIs and p-values will be provided. Covariate variables such as treatment, age and sex will be included.

7.10.3 Clinical laboratory

Haematology, biochemistry and urinalysis are collected at the baseline and end-of-study visits only. For the purpose of summary tables on laboratory test results, any value reported as below the lower limit of quantification or as undetectable will be considered as missing, and any value reported as above the upper limit of quantification will be considered as being equal to the upper limit. In data listings, the reported value will be presented.

For continuous laboratory parameters, summary statistics on the laboratory test results will be presented by visit. For the end-of-study visit, summary statistics on the change from baseline will also be presented.

For categorical laboratory parameters, frequency tables on the laboratory test results will be presented by visit. For the end-of-study visits, the shift from baseline will also be presented.

For all laboratory parameters, frequency tables on the clinical interpretation of the laboratory test results (*e.g.* normal, abnormal but not clinically significant, abnormal and clinically significant) will be presented by visit. For the end-of-study visit, the shift from baseline will also be presented.

Each parameter will be presented in a separate table. A list of all laboratory parameters (including specification of the standardised units to be used for analysis) is presented below:

Clinical laboratory tests will be presented with summary statistics. In addition, the tests will be analysed with ANCOVA (baseline value of respective laboratory test will be included in the model).

All results will be regarded as descriptive only. The p-values will be used to flag safety and tolerability variables worthy of further attention.

Null hypothesis:

- No difference between test treatment and reference treatment

Clinical laboratory tests conducted at planned visits for which laboratory sampling was not planned, as well as unplanned visits with laboratory sampling that are reported through the eCRF, will be included in the listings and, if deemed necessary, commented in the Statistical report.

Laboratory class	Parameter*	Unit**
Haematology	Total red blood cell count	x10^12/L; x10^6/microlitre
	White blood cell count	x10^9/L; x10^3/microlitre ; /microlitre
	Neutrophils	x10^9/L; %; /microlitre
	Lymphocytes	x10^9/L; %; /microlitre
	Monocytes	x10^9/L; %; /microlitre
	Eosinophils	x10^9/L; %; /microlitre
	Basophils	x10^9/L; %; /microlitre
	Haematocrit	L/L; %
	Haemoglobin	Mmol/L; mmolFe/L; g/dL; g/L
	Platelets	x10^9/L; x10^3/microlitre
Biochemistry	Serum creatinine	Micromol/L; mg/dL
	Aspartate aminotransferase	U/L
	Alanine aminotransferase	U/L
	Glucose	Mmol/L; mg/dL
	Serum sodium	Mmol/L; mEg/L
	Serum potassium	Mmol/L; mEg/L
	Total protein	g/L; g/dL
	Total bilirubin	Micromol/L; mg/dL
	Alkaline phosphatase	U/L
	Gamma-glutamyl transferase	U/L
	Cholesterol	Mmol/L; mg/dL
	Triglycerides	Mmol/L; mg/dL
Urinalysis	Specific gravity	
	pH	
	Protein	Positive/Negative
	Glucose	Positive/Negative
	Ketones	Positive/Negative
	Urobilinogen	Positive/Negative
	Erythrocytes	Positive/Negative

	Leucocytes	Positive/Negative

*All measured values are coded as either NCS=Not clinically significant or CS=Clinically significant

** The results of the analyses and data presented in tables will be in SI units.

Test results reported in other units will be converted from their original units to the standardised units specified in the table above before analysis, using appropriate conversion factors. Data listings will include test results in both SDTM, SI and original units, wherever they differ, by using the LBSCAT variable in SDTM.

7.10.4 Vital Signs

Diastolic and systolic blood pressure, pulse rate and body temperature are only measured at baseline, which will be presented in a table. These are annotated as ORRES_DIABP, ORRES_SYSBP, ORRES_PULSE and ORRES_TEMP.

7.10.5 Intraocular Pressure Measurement (mmHg)

IOP is measured at all visits as integer numbers with unit mmHg. The annotation is IOPRES.

7.10.6 Ophthalmoscopy

Ophthalmoscopic assessments are conducted for the vitreous, retina, macula and choroid. Clinically significant findings, reported as *Abnormal, CS*, are to be recorded in the AE log. The annotations are ORRES_VIT, ORRES_RET, ORRES_MAC and ORRES_CHD. The tables can be seen in the DDP.

7.10.7 Worsening of corneal inflammation

This is defined as development of ring abscess and/or hypopyon despite >30 days of treatment with the study drug. The table is presented in the DDP.

7.10.8 Rate of subjects with a relapse

The rate of subjects with relapse will be tabulated, see the DDP. Relapse is recorded in the annotated variable RELAPSYN.

7.10.9 Rate of subjects requiring surgery

Rate of subjects requiring surgery, including amniotic membrane transplants, superficial keratectomy, application of cyanoacrylate glue, therapeutic penetrating, lamellar keratoplasty, cataract surgery, evisceration, or enucleation, will be tabulated, see the DDP. Such surgery will be seen in the annotated variable CMTRT, Name of (prior or concomitant) drug/medication/therapy.

7.10.10 Rate of subjects requiring non-study therapies

Rate of subjects requiring non-study therapies will be tabulated or listed, see DDP. The annotated variable CMTRT will be used as basis.

7.10.11 Rate of subjects' discontinuation from study

The rate of subjects discontinuing the study will be presented, see the DDP. The annotated variable DSCOMP with values *Yes/No* as answer to the question "Did the subject complete the study as per protocol?" will be used. The primary reason for study discontinuation, annotated variable DSDECOD, will also be presented.

The rate of subjects discontinuing the treatment will be presented in a similar fashion, using the DSTRTYN annotated variable, which answers the question "Did the subject complete the treatment as per protocol?". The primary reason for discontinuation, annotated variable DSDECOD, will also be presented. The primary reason for treatment discontinuation, annotated variable DSDECOD_TRT, will also be presented.

7.10.12 Incidence of secondary complication

Incidence of secondary complications, such as significant corneal neovascularization, corneal scarring, corneal perforation, scleritis, secondary glaucoma, cataract, retinopathy will be presented, see the DDP. Such complications will be retrieved from the AE data.

7.10.13 Cure rate of subjects with risk factor

The cure rate of subjects with risk factor defined in section 8.2 will be presented in a frequency table.

7.10.14 Rate of subjects with Adjunctive therapy

The rate of subjects that need Adjunctive therapy will be presented in a frequency table.

Adjunctive therapy is defined as new use after baseline or any increase of at least one of following medication with ATC5:

Topical steroid: dexamethasone, prednisolone, fluorometholone, loteprednol

Oral non-steroidal anti-inflammatories (NSAIDS): diclofenac, ibuprofen, flurbiprofen, naproxen

7.11 Changes to Planned Analysis

At this time, no changes to the planned analyses are being considered.

However, the definition of the PPAS have been altered compared to the protocol due to one wrong sentence.

The use of normal-distribution test assumptions will be the primary choice. If such assumptions should be found incorrect during the analysis of the data, an assessment of which type of analysis that should be used, will be conducted. This will be described in detail in the clinical study report.

8 Derived Variables

8.1 Disposition of Subjects

A screening failure is defined as a screened but not randomised subject.

8.2 Demographics and Baseline Characteristics

Age and sex will be presented using counts, means and rates. Frequency of risk factors present at baseline will also be tabulated. Risk factor is defined as any use of at least one of the following drugs given before baseline with ATC5:

Topical antifungals: amphotericin, voriconazole, chlorhexidine 0.2%, natamycin

Oral antifungals: voriconazole, itraconazole, fluconazole

Topical antivirals: aciclovir, gangciclovir

Oral antivirals: aciclovir, famciclovir, valaciclovir

Topical antibiotics: chloramphenicol, moxifloxacin, levofloxacin, ofloxacin, gatifloxacin, gentamicin, neomycin

Topical steroid potent: dexamathasone 0.1% or Prednisolone forte 1%)

Topical steroid mild: prednisolone 0.5%, FML (flurometha lone), loteprednol

Diagnostic test criteria will be categorised into four (4) groups and be presented in a frequency table:

- Patients diagnosed by IVCM alone: variables CFMPERF and CFMORRES with value *Yes*
- Patients diagnosed by IVCM with culture: variables CFMPERF and CFMORRES with value *Yes* and CSORRES with value *Positive*
- Patients diagnosed by IVCM with PCR: variables CFMPERF and CFMORRES with value *Yes* and PCRORRES with value *Positive*
- Patients diagnosed by IVCM with PCR and culture: variables CFMPERF and CFMORRES with value *Yes* and CSORRES and CFMORRES with value *Positive*

Change from Baseline

Change from baseline will be computed as the difference between a post-baseline value and the corresponding baseline value.

Percentage change from baseline will be computed as 100 times the change from baseline divided by the baseline value.

8.3 Time to Event

The time to clinical resolution in days will be computed as the time in number of days from the start of treatment to the date of resolution or censoring.

For subjects completing the study or being prematurely withdrawn from the study without experiencing clinical resolution, the time to event will be right-censored at the date of completion/withdrawal.

8.4 Compliance

The rate of compliance will be assessed using the *Yes* / *No* answer to the eCRF question: *Was the study drug administration compliant with the protocol?*

8.5 Duration

The time to resolution in days will be computed as the time in days from randomisation to the day of resolution plus 1 day.

9 References

1. ICH Harmonised Tripartite Guideline for Statistical Principles for Clinical Trials E9. February 1998.
2. ICH Harmonised Tripartite Guideline for Structure and Content of Clinical Trial Reports E3. November 1995.
3. Visual acuity measured standard. International Council of Ophthalmolgy, Italian Journal of Ophthalmology II / I 1988, pp 1 / 15.

10 Signoff

We have read this SAP for the 043-SI study and confirm that, to the best of our knowledge, the statistical analyses to be performed in this study are accurately described.

SIFI SpA Approver: Vincenzo Papa MD Ph.D., Medical Affairs Manager

SIGNATURE AND DATE


LINK Medical SAP Author: Christina Ehrenkrona, Sr. Statistician

SIGNATURE AND DATE


LINK Medical SAP Approver: Malin Schollin, Associate Director Biostatistics

SIGNATURE AND DATE


Verification

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Initiator

Intility eSign (Ie)
esign@intility.no

Signing parties

Christina Ehrenkrona (CE)
On behalf of Link Medical Research
christina.ehrenkrona@linkmedical.eu
706040010

Signed 2021-07-01 14:42:42 CEST (+0200)

Vincenzo Papa (VP)
vincenzo.papa@sifigroup.com

Signed 2021-07-02 09:44:50 CEST (+0200)

Malin Schollin (MS)
On behalf of LINK Medical Research AB
malin.schollin@linkmedical.eu
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