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.

Protocol Number: ODAK Phase 3 (043/SI)

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Contract Research Organization	PSR Group BV Crown Business Center Schiphol Planetenweg 5 2132 HN Hoofddorp The Netherlands
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GCP Compliance

This study will be conducted in compliance with Good Clinical Practice, and applicable regulatory requirements.

INVESTIGATOR STATEMENT

I have read amendment 1 to the protocol entitled "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." and the accompanying Investigator's Brochure. I agree to conduct the clinical investigation in compliance with the Final Protocol, the International Conference on Harmonisation (ICH) harmonised tripartite guideline E6(R1): Guideline for Good Clinical Practice, applicable regulatory/government regulations, and in accordance with the latest revision of the Ethical Principles for Medical Research Involving Human Subjects (the Declaration of Helsinki). I will not implement any changes to study procedures or conduct without prior approval from the Sponsor and, when applicable, the Independent Ethics Committee and Regulatory Authority.

I agree to maintain the confidentiality of this study protocol, as described on the title page. Further, I will not publish results of the study without authorization from the Sponsor.

Signature of Principal Investigator	Date

Printed Name of Principal Investigator

Revision History

Version	Description	Rationale for update	Date
001	Final protocol		2017-01-25
002	Protocol amendment 1	Significant changes: treatment of both eyes, corrections of number of visits after relapse. Removal of 0.08% PHMB treatment after End of Study. Administrative updates and textual clarifications.	2018-10-08

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ABBREVIATIONS

AE	Adverse event
AK	*Acanthamoeba* keratitis
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
BCVA	Best corrected visual acuity
CHMP	Committee for Medicinal Products for Human Use
CRF	Case report form (an electronic version is used for this study, eCRF)
CRR	Clinical Resolution Rate
CRR_12	Clinical Resolution Rate at 12 months after randomization, defined as the percentage of subjects cured at 30 days after discontinuing all study therapies within 12 months of randomization
DLE	Dose Limiting Event
DNA	Deoxyribonucleic acid
EMA	European Medicines Agency
EQ-5D	EuroQol five-dimension scale
FAS	Full Analysis Set
FSH	Follicle stimulating hormone
GCP	Good Clinical Practice
HSV	Herpes simplex virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IOP	Intraocular pressure
IRB	Institutional Review Board
IUD	Intrauterine device
IUS	Intrauterine hormone releasing system
LAM	Lactational amenorrhoea method
LAR	Legally authorized representative
NOAEL	No Observed Adverse Effect Level
NSAID	Non-steroidal anti-inflammatory drugs
ODAK	Orphan Drug for *Acanthamoeba* keratitis
PCR	Polymerase chain reaction
PHMB	Polyhexamethylene biguanide
PPAS	Per-Protocol Analysis Set
QPPV	Qualified person responsible for pharmacovigilance
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SOC	System organ class
VAS	Visual analog scale
VFQ25	Visual functioning questionnaire 25

STUDY SYNOPSIS

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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.

DEFINITIONS

Protocol section 2.1 Definitions, page 28

Clinical resolution is a clinical definition, resulting from a 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 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.

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. This is the primary outcome measure. Subjects whose disease has not resolved at 12 months are considered treatment failures per protocol.

OBJECTIVES

Protocol section 2.2 Objectives, page 28

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 with that of 0.02% PHMB + 0.1% propamidine combination therapy, 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.

A further aim of this study is to obtain additional safety information on 0.08% PHMB ophthalmic solution.

HYPOTHESIS

Protocol section 2.3 Hypothesis, page 28

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 below, 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/SI) (63% when assuming a prevalence of late stage diseases in 38% of subjects).

OUTCOME MEASURES (Efficacy and Safety Parameters)

Protocol section 6.3 Primary Efficacy Variables, page 55
Protocol section 6.4 Secondary Variables, page 56

The primary efficacy parameter chosen to assess drug efficacy is the CRR_12.

Secondary efficacy parameters are:

- Best corrected visual acuity (BCVA)
- Corneal scarring as identified by slit lamp examination
- Ulceration severity as identified by slit lamp examination using a 2 grade scoring procedure
- Anterior chamber inflammation as identified by ophthalmoscopy using a 3 grade scoring procedure
- EQ-5D questionnaire
- VFQ25 questionnaire

Secondary safety parameters are:

- Adverse events
- 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
- 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.

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

OVERVIEW OF STUDY DESIGN

Protocol section 3.1 Overview of Study Design, page 29

This is a randomized, assessor-masked, active-controlled, multiple center, 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.

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

A total of 130 subjects affected by *Acanthamoeba* keratitis will be assigned to one of the following 2 treatment groups in a ratio of 1:1.

Group 1: 0.08% PHMB + placebo

Group 2: 0.02% PHMB + 0.1% propamidine combination therapy

SUBJECT POPULATION

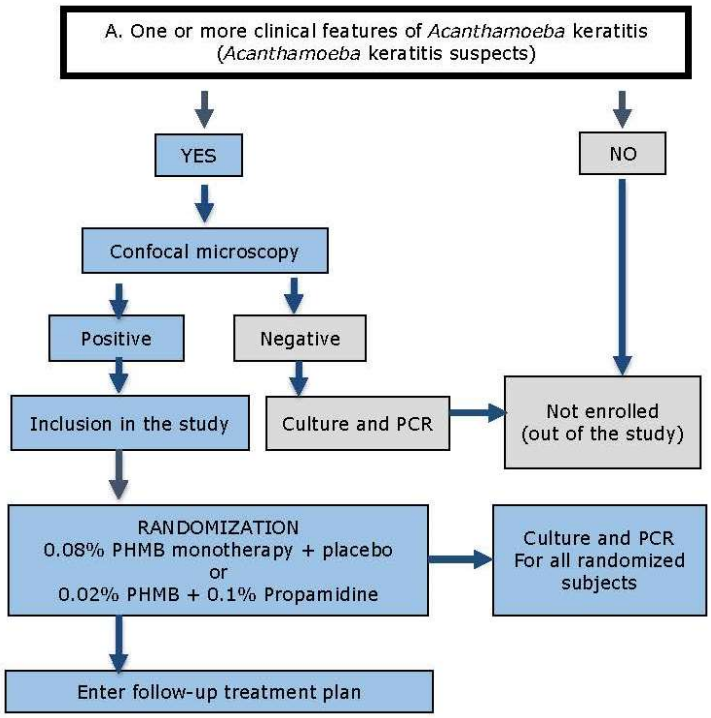
Protocol section 4, SUBJECT POPULATION*, page 33*

The study will be performed in male and female subjects affected by *Acanthamoeba* keratitis, ≥12 years of age, inclusive.

SCREENING PROCEDURE

Protocol section 3.1.2 Screening Visit, page 30

A schematic overview of the screening procedure is presented below.



All subjects with clinical signs consistent with *Acanthamoeba* keratitis **and** positive confocal findings consistent with *Acanthamoeba* keratitis will be eligible for randomization providing they have not been using a topical biguanide or diamidine.

All subjects at screening, meeting inclusion criteria **and** including those with negative confocal microscopy findings will have corneal scrapes for culture and histology of *Acanthamoeba*, and to perform *Acanthamoeba* deoxyribonucleic acid (DNA) identification by polymerase chain reaction (PCR).

Note: this is being done for study quality, for correlation with confocal microscopy findings, and to identify patients with fungal and viral infection. Because culturing and PCR will take 2 to 8 days, during which time subjects thought clinically to have *Acanthamoeba* keratitis would be starting treatment before randomization, this assessment is not part of the inclusion criteria. Subjects who have been proven to have fungal and viral infections, if randomized on the basis of clinical and confocal microscope findings, will be removed from the study, even though they may have concomitant *Acanthamoeba* keratitis.

Subjects with a negative confocal microscopy evaluation but a positive PCR/Culture (confocal false negative) will **not** be enrolled in the study but treated with the best clinically available treatment outside the study.

Subjects meeting the inclusion criteria, including findings on slit lamp examination and confocal microscopy consistent with *Acanthamoeba* keratitis are entered into the study and randomized to one of the two treatment arms. They will then enter the Treatment Plan and Follow-up protocol as presented on page 16.

INCLUSION CRITERIA

Protocol section 4.1 Inclusion Criteria, page 33

Subjects must meet all of the following criteria to be considered eligible to participate in the study:

1. Subject must be able and willing to give informed consent.
2. Subject must be a man or woman of any race and ≥12 years of age, inclusive. Subjects <18 years will only be enrolled in selected study sites
3. Subject must be able to understand and willing to comply with study procedures, restrictions and requirements, as judged by the investigator.
4. Clinical findings consistent with *Acanthamoeba* keratitis

   Clinical findings include the following:

   - Epithelial lesions: epithelial punctate keratopathy, epithelial infiltrates, epithelial defects, dendritiform epithelial ulcers.
   - Extracorneal lesions: limbal inflammation (limbitis), anterior scleral inflammation, diffuse or nodular.
   - Stromal lesions: perineural infiltrates, anterior stromal infiltrates, disciform corneal swelling, stromal ulceration, ring abscess.
   - Anterior chamber lesions: keratic precipitates, hypopyon.
   - Late findings: fixed dilated pupil, mature cataract
5. Confocal microscopy findings consistent with *Acanthamoeba* keratitis (performed within 7 days prior to study entry or as part of screening procedures)
   - Confocal microscopy findings include: cysts are round or ovoid, may show a double wall and are 15-30 μm in size.
6. Subjects using the following previous treatments for *Acanthamoeba* keratitis are eligible for the study:
   - Antibiotics: subjects who have an ocular bacterial infection at baseline are eligible for the study. However, only topical moxifloxacin is permitted, unless resistant or contraindicated.

     Note: subjects who develop intercurrent bacterial infections will be retained in the study and treated with topical moxifloxacin.
   - Antiviral drugs and antifungal drugs: subjects are often misdiagnosed as having these infections when they have *Acanthamoeba* keratitis. Subjects taking antivirals and antifungal agents (except for any using PHMB or Chlorhexidine) for a misdiagnosis can be included, but must discontinue these drugs after entry into the study.

     Note: subjects who are thought to have combined *Acanthamoeba* keratitis with herpes or fungal keratitis are excluded from the study.
   - Anti-inflammatory drugs: Subjects using topical steroids and/or oral NSAIDs before the diagnosis of *Acanthamoeba* keratitis are eligible for the study. However, these subjects must agree to change therapy to the topical steroids and oral NSAIDs that are specified for use in the study (see Concomitant and Previous Medications).
7. Females of childbearing potential will be included if they are either sexually inactive (sexually abstinent for 14 days prior to the first study drug dose continuing through 28 days after the last study drug dose, or using one of the following highly effective contraceptive (i.e. results in <1% failure rate when used consistently and correctly) methods in this study:
   a. intrauterine device (IUD);
   b. surgical sterilization of the partner (vasectomy for 6 months minimum);
   c. combined (estrogen or progestogen containing) hormonal contraception associated with the inhibition of ovulation (either oral, intravaginal, or transdermal);

d. progestogen only hormonal contraception associated with the inhibition of ovulation (either oral, injectable, or implantable);

e. intrauterine hormone releasing system (IUS);

f. bilateral tubal occlusion.

Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. In this study, abstinence is only acceptable if in line with the subjects preferred and usual lifestyle.

Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM) are not acceptable methods of contraception. As well, a female condom and a male condom should not be used together.

8. Females of childbearing potential agree to remain sexually inactive or to keep the same birth control method for at least 28 days following the last study drug dose.

9. A female of non-childbearing potential must have undergone one of the following sterilization procedures at least 6 months prior to the first study drug dose:

   a. hysteroscopic sterilization;

   b. bilateral tubal ligation or bilateral salpingectomy;

   c. hysterectomy;

   d. bilateral oophorectomy;

   or be postmenopausal with amenorrhea for at least 1 year prior to the first study drug dose and follicle stimulating hormone (FSH) serum levels consistent with postmenopausal status

10. A non-vasectomized male subject agrees to use a condom with spermicide or abstain from sexual intercourse during the study until 90 days beyond the last dose of study drug and the female partner agrees to comply with inclusion 7 or 8. For a vasectomized male who has had his vasectomy 6 months or more prior to study start, it is required that they use a condom during sexual intercourse. A male who has been vasectomized less than 6 months prior to study start must follow the same restrictions as a non-vasectomized male.

    Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and LAM are not acceptable methods of contraception. As well, female condom and male condom should not be used together.

11. If male, they must agree not to donate sperm from the first study drug dose until 90 days after dosing.

EXCLUSION CRITERIA

Protocol section 4.2 Exclusion Criteria, page 35

Subjects who meet any of the following criteria will be excluded from participation in the study:

1. Subject with documented history and/or clinical signs of concomitant presence of an ocular infection caused by viruses (herpes simplex virus [HSV]) or fungi.
2. Subject treated with drugs having effects on *Acanthamoeba* cysts prior to study entry, including biguanides (PHMB, chlorhexidine) and diamidines (propamidine, hexamidine).
3. Subjects requiring systemic immunosuppression for *Acanthamoeba* associated scleritis.
4. Subjects requiring urgent surgical intervention for advanced *Acanthamoeba* keratitis in either eye (e.g., for advanced corneal thinning/melting etc.).
5. Subject with known or suspected allergy to biguanides, diamidines or intolerance to any other ingredient of the investigational treatments.
6. Subject affected by immunodeficiency diseases or taking systemic immunosuppressive therapy.

7. Subject with a major systemic disease or other illness that would, in the opinion of the investigator, compromise subject's safety or interfere with the collection or interpretation of study results.
8. If female, pregnancy, planned pregnancy, or breast-feeding
9. Subject is participating in another interventional clinical study with an experimental or unapproved/unlicensed therapy or has participated in another interventional clinical study within 4 weeks prior to this study.

The investigator must ensure that all study enrolment criteria have been met at randomization. If a subject's status changes after randomization, but before the first dose of study drug is given such that he or she no longer meets all eligibility criteria, then the subject should be excluded from participation in the study.

CONCOMITANT AND PREVIOUS MEDICATIONS

Protocol section 4.3 Concomitant Medication and Other Restrictions during Study, page 36

Subjects are allowed to use the following concomitant medications during the study:

Antibiotics: Topical moxifloxacin is permitted for the treatment of intercurrent bacterial infections (unless culture and sensitivity, or clinical progress demands a change). Topical moxifloxacin is not permitted for use as a prophylactic antibiotic in patients with corneal ulcers; PHMB is a good broad spectrum anti-bacterial and an additional antibacterial is not needed for this. The value of prophylaxis is unproven.

Antiviral drugs and antifungal drugs: The use of these drugs is *not* allowed during the study. In case subjects use antiviral or antifungal drugs at study entry, they must be discontinued.

Anti-inflammatory drugs: For subjects on steroids at study entry (already using topical steroids for e.g. mistaken diagnosis of HSV keratitis or as adjunctive treatment for bacterial keratitis), there are the following options:

a. Either stop steroids, OR maintain, OR reduce the doses (at the investigator's discretion). Unpreserved Dexamethasone (0.1% or 0.15%) is the only topical steroid permitted for use in this trial. Patients using any other topical steroids on trial entry should be changed to this at the appropriate frequency. Diclofenac is the only oral NSAID permitted in the trial and will be ADDED at the appropriate dose (75 mg to 150 mg daily, divided in two or three doses) and continued at any level while topical steroids are in use during the study.

b. Subjects using topical NSAIDs and ciclosporin at study entry should have these discontinued after randomization.

c. Subjects using no topical steroids at study entry can have these started together with oral NSAIDs (recommended diclofenac; 75 mg to 150 mg daily, divided in two or three doses) during the study as specified in the schematic overview of the Treatment and Follow-up Protocol.

Other permitted topical mediations: Unpreserved lubricants, mydriatics (cyclopentolate, homatropine or atropine) and glaucoma medications are permitted.

TREATMENT ASSIGNMENT

Protocol section 5.1 Treatment Assignment , page 38

Randomization to one of the treatment groups will be in a 1:1 ratio. The randomization schedule will be generated using a computer program and verified for accuracy using strict quality control procedures. Eligible patients will receive a masked treatment assignment with a unique randomization code based on the randomization list. The assigned randomization code will be captured in the electronic Case Report Form (eCRF).

Protocol for bilateral disease: if both eyes are affected, only one eye will be treated with the study treatment and will be considered for the study. This will be the right eye, unless the severity of the infection differs in both eyes, in which case the worst affected eye should be selected. The fellow eye should be treated with the best treatment according to clinical practice.

DRUG DOSAGE AND ADMINISTRATION

Protocol section 5.2 Drug dosage and Administration, page 39

On Day 0, subjects will receive instructions on how to apply the eye drops. Subjects will receive the assigned treatment

to be administered in the affected eye until resolution. Subjects will be treated for a maximum of 1 year after randomization and subjects meeting the criteria for clinical resolution at or before 12 months will be followed up for 90 days before completing the study.

Subjects will receive an initial intensive course of 0.08% PHMB + placebo or 0.02% PHMB + 0.1% propamidine combination therapy according to the following schedule. Subjects will be treated for a maximum of 1 year after randomization.

Treatment throughout the study will be given using 1 drop of each ophthalmic solution in the study eye at different frequencies during the daytime only. At study entry, an intensive 19-day antiamoebic treatment protocol will be initiated as follows:

Day 0 to 5	1-hourly drops (16 drops in a day) for 5 days
Day 6 to 12	2-hourly drops (8 drops in a day) for 7 days
Day 13 to 19	3-hourly drops (6 drops in a day) for 7 days
Day 20	Then 4x daily thereafter

Notes on treatment

i. The frequency will be maintained at 4x daily unless there is a relapse (see Box XI, relapse, Figure 2 on page 16). In the event of a relapse, culture negative subjects will receive the same intensive 19-day treatment course as given at study entry.

ii. Antiamoebic drops will be discontinued when clinical resolution is reached (see Figure 2 and Legend of BOX IV DISCONTINUE TREATMENT on page 16).

iii. Clinical resolution is a clinical definition, resulting from a 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 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.

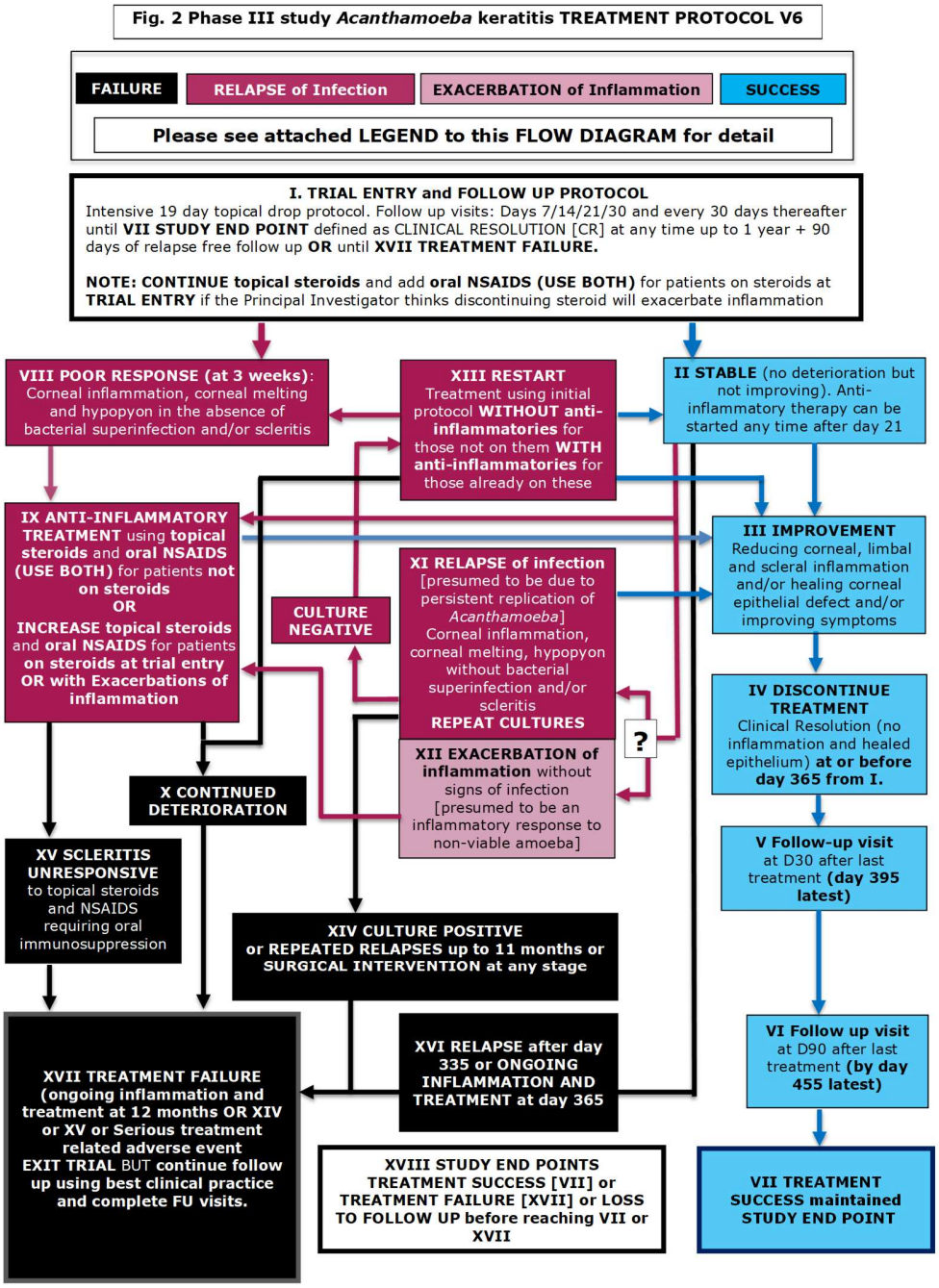
For subjects using steroids at study entry (already using topical steroids for e.g. mistaken diagnosis of HSV keratitis or as adjunctive treatment for bacterial keratitis), the same intensive 19-day antiamoebic treatment protocol will be used. For their steroid use there are the following options:

- Either stop steroids, OR maintain, OR reduce the doses (at the investigator's discretion). Unpreserved Dexamethasone (0.1% or 0.15%) is the only topical steroid permitted for use in this trial. The frequency of use is at the discretion of the investigator.
- Patients using any other topical steroids on trial entry should be changed to this at the appropriate frequency. Diclofenac is the only oral NSAID permitted in the trial and will be ADDED at the dose determined by the investigator (75 mg to 150 mg daily, divided in two or three doses) and continued at any level while topical steroids are in use during the study.

Subjects using antifungals or antivirals on study entry must discontinue these after randomization.

SCHEMATIC OVERVIEW OF THE TREATMENT AND FOLLOW-UP PERIOD

Protocol section 5.3 Treatment and Follow-up Protocol, page 41



. Legend for Figure 2: Schematic Overview of the Treatment and Follow-up Period

The pathways are **colour coded**:

- **Pale blue text boxes and lines** for responding disease or treatment success and **STUDY END POINT**
- **Red text boxes and lines** for poorly responsive and recurrent / relapsing disease presumed to be due to replication of *Acanthamoeba*
- **Pink text box for** exacerbations of inflammation presumed to be due to flare ups of inflammation secondary to the immune response to non-viable *Acanthamoeba*
- **Black text boxes** for treatment failure endpoints ALL leading to **TRIAL EXIT**

Each box is labelled with a Roman numeral I to XVIII. The legend below describes the rationale for each step:

I. FOLLOW UP PROTOCOL from day zero at TRIAL ENTRY.

Treatment throughout the study is given using 1 drop of each ophthalmic solution in the study eye at different frequencies during the daytime only:

At trial entry initiate an intensive 19 day anti-amoebic treatment protocol:

Day 0-5: every hour daytime only hourly drops (16 drops a day) for five days.

Day 6-12: 2 hourly (8 drops a day) for 7 days

Day 12-19: 3 hourly (6 drops a day) for 7 days

Day 20: reduce to 4x daily and continue thereafter.

a. This frequency is maintained at 4x daily unless there is a relapse of infection (XI RELAPSE of infection) when culture negative patients receive the same intensive 19 day treatment course as given at trial entry.

b. Anti-amoebic drops are discontinued as when the clinical findings are as described in IV DISCONTINUE TREATMENT.

In patients on steroids at TRIAL ENTRY, (already using topical steroids for e.g. mistaken diagnosis of herpes simplex virus keratitis or as adjunctive treatment for bacterial keratitis) the same intensive 19 day anti-amoebic treatment protocol is used, there are the options:

Either stop steroids, OR maintain, OR reduce the doses (at the Principal Investigator's discretion). **ADD an oral non-steroidal anti-inflammatory drug (NSAID) WHEN steroid is to be continued** at any level.

In patients using antifungals or antivirals on trial entry these must be discontinued.

II. STABLE (defined as no deterioration but not improving, as opposed to a poor response as defined in VIII). These subjects may improve (meeting the criteria described in Box III IMPROVEMENT: see below) or not over the next few weeks. If there is no improvement but they remain stable, then these patients may be started on anti-inflammatory treatment as described in IX. Alternatively, the **inflammation may deteriorate** (this may occur at any stage of treatment) and the rationale for managing this problem is described here:

DETERIORATION of inflammation (after excluding co-infection with other organisms).
The two possibilities which the PI needs to differentiate between are:

a. Relapse of infection (Box XI) presumed or proven to be due to continued replication of *Acanthamoeba*) OR
b. Exacerbation of inflammation (Box XII) presumed to be due to inflammation secondary to the immune response to non-viable *Acanthamoeba*

XI **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. This is as opposed to:

XII **EXACERBATION of inflammation:** which is accompanied by mild conjunctival or corneal inflammation (sometimes coarse anterior stromal infiltrates like those following adenovirus keratitis) and/or mild scleritis. XV SCLERITIS unresponsive to topical steroids and oral NSAIDS is another cause of this: these patients are complex to manage and as a result we decided that these should exit the trial.

Once a decision has been made between these two the appropriate protocol is followed for each scenario as outlined in XI and XII.

Note: if this occurs after 11 month (335 Days) it is a treatment failure XVII (as patients relapsing at this point will not have time to respond to treatment) and the patient exits the trial.

III. IMPROVEMENT reducing inflammation and some healing of any ulcers and/or improving symptoms. These subjects may continue to improve to the point they go to IV Discontinue treatment.

Deterioration of inflammation: Patients in this subset may deteriorate due to: XI Relapse of Infection or XII Exacerbation of inflammation in which case they are managed as described in paragraph II a, b for these issues.

IV. DISCONTINUE TREATMENT (includes topical steroids, oral NSAIDs and topical anti-amoebics/microbials): when any ulcers have healed, and all signs of AK related inflammation have resolved (see detailed description in protocol, mild conjunctival inflammation related to drugs or other condition such as blepharitis are acceptable) at or before 12 months (365 days) from Trial entry. Clinical resolution (CR) after 365 days is a failure (see Box XVI). We recommend discontinuing topical steroids 2-4 weeks before discontinuing topical anti-amoebics.

V. Follow up visit at Day 30 after last treatment.

Deterioration of inflammation: Patients in this subset may deteriorate due to: XI Relapse of Infection or XII Exacerbation of inflammation in which case they are managed as described in paragraph II a, b for these issues.

VI. Follow up visit at Day 90 after last treatment. If clinical resolution has been maintained by 15 months (day 455 at the latest) these are treatment successes and have reached the study endpoint (Box VII).

VII. TREATMENT SUCCESS.

VIII. POOR RESPONSE at 3 weeks from I Trial entry or XIII RESTART: patients are managed using the guidelines in IX (this is the earliest that anti-inflammatory treatment can be started for those not on this when included in the Trial). Criteria for a poor response are described in detail in the protocol and include enlarging ulcer, stromal thinning, deteriorating corneal inflammation (infiltrate including ring abscess), intraocular inflammation (uveitis and hypopyon) and/or scleritis.

IX. ANTI-INFLAMMATORY TREATMENT

a. For patients NOT using topical steroids start these WITH oral NSAIDS (this is requested to simplify anti-inflammatory treatment - rather than ad hoc switching).
b. For patients already on topical steroids and oral NSAIDS, increase the potency or frequency of the steroids and NSAIDS.

If this results in III. IMPROVEMENT the management continues down the blue pathway.

X. Alternatively, if CONTINUED DETERIORATION occurs cases move to XVII TREATMENT FAILURE and EXIT Trial (see below).

XI. RELAPSE of Infection within 11 months, after an initial response is common. Cultures for *Acanthamoeba* and other organisms (fungi bacteria and herpes) must be repeated. Carry out a corneal biopsy, if PI thinks this is appropriate.

a. If the CULTURES are NEGATIVE patients this may mean that the anti-amoebic therapy is having some effect although we have no way of being certain about this (confocal and PCR don't help as these can be positive when the organisms are non-viable). These patients are managed as described in XIII below with another 19 day course of intensive therapy, with or without anti-inflammatory therapy depending on status.

b. If CULTURES are POSITIVE patients have TREATMENT FAILURE and managed as described in XIV and XVII below.

XII. EXACERBATION of inflammation is presumed to be due to inflammation secondary to the immune response to non-viable *Acanthamoeba* and anti-inflammatory treatment can be re-started or increased at the discretion of the PI without the need for re-culture and a further course of intensive anti-amoebic therapy. **IMPORTANT**: if anti-amoebic treatment had been stopped restart this when anti-inflammatory treatment is re-started.

Distinguishing between XI and XII is often difficult and guidelines for this are given in II a, b above.

XIII. Restart with another 19 day intensive course of anti-amoebic therapy with or without topical steroids and oral NSAIDS:

a. If patients were not on these at the time of the relapse of infection we recommend not using these until after the 19 day course of intensive treatment and depending on response move to as above.

b. If they were on them at the time of the relapse continue at a dose determined by the PI and follow pathways II, III, IX or X.

XIV. CULTURE POSITIVE these patients are Exit Trial and are managed as described in XVII below.

XV. SCLERITIS UNRESPONSIVE to treatment in IX. This is a failure (see XVII below).

XVI. RELAPSE after day 335 or ingoing inflammation and treatment at day 365: this is a treatment failure and is managed as described in XVII below

XVII. TREATMENT FAILURE and EXIT TRIAL.

XVIII. Summary of Study end points.

STUDY EVALUATIONS

Protocol section 6 STUDY EVALUATIONS, page 51

Efficacy evaluations will consist of assessment of best corrected visual acuity (BCVA), pupil test (swinging light test), slit lamp examination, EuroQol five-dimension scale (EQ-5D) questionnaire, and visual functioning questionnaire 25 (VFQ25).

Safety and tolerability evaluations will consist of adverse event (AE) reporting, clinical laboratory (hematology, biochemistry and urinalysis), intraocular pressure (IOP), ophthalmoscopy, and photography of affected cornea.

Detailed information about adverse event reported is provided in Section 0.

See the Schedule of Assessments on page 22, Section 6 and Appendices 1 to 3 in the full protocol, and the Study Operations Manual for details of the evaluations.

SUBJECT COMPLETION/WITHDRAWAL

Detailed information is provided in Section 4.4 of the full protocol

STATISTICAL METHODS

Protocol section 7 STATISTICAL METHODS, page 57

A statistical analysis plan (SAP) with more technical and detailed elaboration of the principal features of the proposed statistical analysis, presentations and the way in which anticipated analysis problems will be handled, will be written before the code is broken. See Section 7 for detailed information.

Sample Size Determination

From the results of the sponsor's observational, case series retrospective study 038/SI, a CRR_12 of 67% for the conventional combination therapy 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 favor 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 diseases 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).

Efficacy

All efficacy evaluations will be based on the Full Analysis Set. Moreover, the primary efficacy variable, CRR_12, will be evaluated also for the Per-Protocol Analysis Set.

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% confidence interval for the difference between the treatments obtained from the superiority analysis gives the necessary information. Position of the lower end of the confidence interval relative to a pre-defined agreed 0.20 non-inferiority margin (Δ) provides the key information for making decisions (conclusions) about 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 Full Analysis Set (FAS) and the Per-Protocol Analysis Set (PPAS), giving similar results with respect to p-values and confidence intervals 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 (CPMP) 482/99.

Null hypothesis superiority:

- 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 analyzed with a logistic regression model and model-based estimates of the difference between treatments, odds ratio with corresponding confidence intervals and p-values will be provided. Time-to-event (clinical resolution) analysis will also be performed for the comparison of CRR_12s between treatments, including 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.

Which test to be used in the analyses of secondary efficacy variables depends on the type of variable, e.g., logistic regression for binary variables, proportional odds model for an ordinary scaled variable, analysis of variance (ANOVA) or covariance (ANCOVA) for continuous variables as appropriate. If the underlying assumptions are not fulfilled, data transformation or non-parametric test will be performed. For comparison of proportions, Chi square tests, Fisher's exact test, or Mantel-Haenszel procedures, as appropriate, will be considered. For continuous variables, the Wilcoxon rank-sum test or rank ANOVA/ANCOVA will be used. The STATA or SAS statistical software will be used for the analysis.

EQ-5D and VFQ25 will be evaluated according to their respective user protocols.

Safety

AEs data will be captured and encoded by system organ class (SOC) and 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.

Clinical laboratory tests will be presented with summary statistics. In addition, the tests will be analyzed 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 hypotheses:

- No difference between test treatment and reference treatment.

QUALITY CONTROL AND QUALITY ASSURANCE

Detailed information is provided in Section 9 of the full protocol.

ETHICAL ASPECTS

Detailed information is provided in Section 10 of the full protocol.

SCHEDULE OF ASSESSMENTS

Visits [1]	Screening	Treatment Period						Follow-up Period		Relapse [3]	End-of-study [4]
Study Procedure **Study Day**	**-2 to 0 [5]**	**0**	**7 ± 2**	**14 ± 2**	**21 ± 2**	**30 ± 3**	**60 ± 3 and every 30 ± 5 until resolution**	**30 ± 3 after end of treatment**	**90 ± 5 after end of treatment[2]**		
Screening/Administrative/Baseline											
Informed consent	x										
Parent(s), legal guardian(s), or legally authorized representative (LAR) Informed consent [6]	x										
Demographics	x										
Review medical history [7]	x										
Inclusion/exclusion criteria	x										
Clinical suspicion of an *Acanthamoeba* infection by typical clinical findings	x										
Confirmation of *Acanthamoeba* infection by confocal microscopy [8]	x										
Corneal scraping [9]	x									x	
Vital signs [10]	x										
Study Drug Administration											
Randomization [11]		x									
Dispense study drug [12]		x				x	x			x	
Study drug administration [13]		x	x	x	x	x	x			x	
Efficacy Assessments											
Best corrected visual acuity		x[11]									x
Slit lamp examination		x[11]	x	x	x	x	x	x	x	x	x
Pupil test (swinging light test) [14]		x[11]	x	x	x	x	x	x	x	x	x

Visits [1]	**Screening**	**Treatment Period**						**Follow-up Period**		**Relapse** [3]	**End-of-study** [4]
Study Procedure Study Day	**-2 to 0** [5]	**0**	**7 ± 2**	**14 ± 2**	**21 ± 2**	**30 ± 3**	**60 ± 3 and every 30 ± 5 until resolution**	**30 ± 3 after end of treatment**	**90 ± 5 after end of treatment**[2]		
EQ-5D questionnaire [15,16]		x[11]	x	x	x	x	x	x	x	x	x
Visual functioning questionnaire (VFQ25) [15,17]		x[11]	x	x	x	x	x	x	x	x	x
Safety Assessments											
Hematology, biochemistry	x										x
Urinalysis (dipstick)	x										x
Urine pregnancy dipstick (women of childbearing potential only	x					x	x	x	x	x	x
Use of topical steroids and oral NSAIDs [18]	x				x						
IOP		x[11]	x	x	x	x	x	x	x	x	x
Ophthalmoscopy [19]		x[11]									x
Photography of affected cornea		x[11]				x			x		x
Ongoing Subject Review											
Concomitant therapy [7]	x	x	x	x	x	x	x	x	x	x	x
Adverse events	x	x	x	x	x	x	x	x	x	x	x

1. During each visit, subjects will enter the clinical research center and will leave after completion of all assessments.
2. If subjects are cured 90 days after end-of-treatment visit, the study-end-point is reached and the End-of-Study assessments will be performed.
3. If within 90 days after end-of-treatment and within 11 months (Day 335) after randomization the subject relapses, and cultures are negative for *Acanthamoeba*, treatment with the study drug will be re-started according to the treatment schedule, starting with the Day 0 treatment regime. Assessments and visits will also be restarted at Day 0, Day 30 and all consecutive visits of the treatment phase. A detailed treatment plan and follow-up protocol is provided in Section 5.3.
4. End-of-study is defined as: (a) the last follow-up visit at 90 days after end of treatment, (b) the day of study drug unmasking in the event of the subject becoming a Treatment failure, (c) if withdrawn from the trial of other reasons, (d) if a subject has been treated for 12 months after randomization (also a Treatment failure).
5. Screening and baseline assessments will take place on Day -2 to 0, before randomization and study drug administration.
6. For subjects who have not reached the age of majority (i.e. 18 years of age in most jurisdictions), the subject must provide assent (written or verbal) and the parent/guardian/legally authorized representative must provide written informed consent before the subject's participation in the study.
7. All medical and medication history related to the current eye infection will be reported. For the remaining medical and medication history, only the last 30 days before signing the ICF will be reported in the case report form (CRF).
8. Confocal microscopy is optional if already performed within 7 days prior to study entry as part of standard care providing the images are available to the Investigator to assess.

9. All subjects at screening, meeting the inclusion criteria and including those with negative confocal microscopy findings will have corneal scrapes for culture and histology of *Acanthamoeba*, and to perform *Acanthamoeba* deoxyribonucleic acid (DNA) identification by polymerase chain reaction (PCR). Note: this is being done for study quality, for correlation with confocal microscopy findings, and to identify subjects with fungal and viral infections. Because culturing and PCR will take 2 to 8 days, during which time subjects thought clinically to have *Acanthamoeba* keratitis and would be starting treatment before randomization, this assessment is not part of the inclusion criteria. This assessment is optional if already performed within 7 days prior to study entry as part of standard care. Corneal scraping for culture and histology of *Acanthamoeba*, and to perform *Acanthamoeba* DNA identification by PCR will be repeated in case of a relapse within 90 days after end-of-treatment and within 11 months (Day 355) after randomization.
10. Blood pressure, pulse rate and body temperature.
11. Before first study drug application.
12. Study drug will be provided for 30 days at the Day 0 visit. At other dispensing visits, study drug will be provided for 90 days of treatment.
13. On Day 0, the first study drug application will be at the clinical research center. Thereafter, study drug will be self-administered at home. When the subject is at the clinical research center for assessments, subjects will apply the study drug themselves at the clinical research center. 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.
14. The Pupil test (swinging light test), to detect an afferent pupillary defect, is used to detect retinal or optic nerve involvement when the retina cannot be examined.
15. If the subject is unable to complete the questionnaires without assistance, the questionnaires may be verbally answered and the subject's responses recorded by site personnel or a chaperone. When multiple assessments are scheduled at the same time, completion of questionnaires by the subject will be done before the clinical examinations.
16. Subjects being ≤15 years of age will complete the youth version of this questionnaire.
17. Only to be performed by subjects of ≥18 years.
18. The use of topical steroids and oral NSAIDs is complex and described in the Synopsis and Protocol. For subjects not already using topical steroids or NSAIDs on trial entry these may be introduced at day 21 in subjects whose inflammatory signs are not improving. Please see the Synopsis and Protocol for detailed guidelines.
19. Ophthalmoscopy will be performed at baseline on all patients and at any time thereafter if the pupil tests or intraocular pressure are abnormal.

1. 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.

1.1. Background

PHMB is a poly-cationic polymer composed by hexamethylene biguanide units (n varies 2 to 40 with a mean of 5.5).[1] Biguanides are an important class of cationic surface-active antimicrobial agents, which have been used for the preservation of many aqueous formulations in addition to the use as disinfectants and antiseptics. PHMB is currently used as an environmental biocide and antiseptic in a variety of products including wound care dressings, contact lens cleaning solutions, perioperative cleansing products and swimming pool cleaners. It has a broad spectrum of activity, being effective against gram-positive and gram-negative bacteria. At a cellular level in *Escherichia coli,* PHMB interacts with the cytoplasmic membrane, causing leakage of cellular components and inhibition of respiratory enzymes considered essential for survival.[1,2,3]

PHMB has been shown to have excellent *in vitro* activity against a broad range of fungal pathogens. Antimicrobial effectiveness has been demonstrated on *Acanthamoeba polyphaga*, *Acanthamoeba castellanii* and *Acanthamoeba hatchetti*. Against these protozoa, PHMB acts by binding of its highly charged positive molecules to the mucopolysaccharide plug of the ostiole. This results in penetration through the ostiole to the internalized amoeba, where the drug binds to the phospholipid bilayer of the amoeba cell membrane causing membrane damage, cell lysis and death.[4] PHMB is effective and well tolerated at concentrations of 200 to 600 mg/L (0.02%-0.06%) when used as treatment of patients with *Acanthamoeba* keratitis.[5,6]

1.1.1. Toxicology

PHMB is considered safe when used as preservative in cosmetics up to a concentration of 0.3%.[7] In addition, PHMB is considered of moderate acute oral toxicity with a no observed adverse effect level (NOAEL) of 36 mg/kg/bw.[7] Despite the weak evidence of the carcinogenetic potential, PHMB is classified in the category "Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential" for inciting vascular tumors in laboratory animals by the oral and dermal routes.[7] No quantitative or qualitative susceptibility was evident in the available prenatal developmental toxicity studies. Moreover, a 2-generation reproductive study did not show quantitative or qualitative evidence of increased susceptibility to abnormalities in the offspring when adults were exposed to PHMB. No mutagenic or genotoxic responses were observed.[7] At ocular level, pure or powder PHMB induced irreversible ocular damage and was considered as corrosive to the rabbit eye, whereas a 20% solutions induced conjunctivitis, corneal opacity, iritis and was considered as moderately irritating to the eye.[7] Concentrations of 0.05% and a 0.1% PHMB solution have been tested in an irritation test on enucleated pig eyes without causing

desquamation of epithelial cells or damage to the microvilli. In addition, the administration of a 0.2% PHMB 8 times a day for 4 weeks into the conjunctival sac of the rabbit eye caused mild transient conjunctival redness and discharge. However, the above toxicity signs were not observed if administered twice daily. Accordingly, PHMB (0.1% and 0.2%) was categorized as non-irritant (Draize test) after a twice daily application over a period of 28 days in the rabbit.[8] A repeated intensive ocular administration in rabbits of 0.08%, 0.25% and 0.8% PHMB for 2 weeks showed no toxicity of PHMB up to 0.25%. Only PHMB at 0.8% induced moderate/severe treatment-related and irreversible effects.[9] The safety of 0.08% PHMB following repeated ocular administration to the rabbit over a period of 26 weeks was also investigated. The treatment regimen was 16 times/day at approximately 1 hour intervals from Day 1 to 5 of the study, 8 times/day at approximately 2 hours intervals from Day 6 to Week 3 and 4 times/day at approximately 4 hours intervals from Week 4 to 26. The results did not report any significant findings with the exception of a light conjunctival redness, a slight conjunctival chemosis and slight discharge during the first 4 days. No fluorescein staining has been observed. No treatment-related changes were reported at the histopathological examination of the eyes, adnexa or in the remaining organs/tissues.[9] For additional non-clinical information, refer to the Investigator's Brochure for PHMB.[10]

1.1.2. Clinical Studies

To date, the use of PHMB has been shown to be well tolerated and safe in ophthalmology. Moreover, 0.02% PHMB topical toxicity has been reported rarely and it is limited to mild punctate keratitis.[11]

A prospective, randomized comparative study was conducted to compare the therapeutic efficacy of two agents, PHMB and chlorhexidine, in the treatment of *Acanthamoeba* keratitis. According to the Cochrane Database Review, this is to date the only randomized, controlled study conducted in patients with *Acanthamoeba* keratitis.[12] Fifty-six eyes of 55 patients with *Acanthamoeba* keratitis were randomized to receive 0.02% PHMB or 0.02% chlorhexidine. Treatments were started immediately after a clinical diagnosis of *Acanthamoeba* keratitis was established. PHMB 0.02% or chlorhexidine 0.02% was commenced at a frequency of hourly day and night for the first two days, then frequency was reduced to hourly by day only for the next five days. The frequency of instillation was further reduced to four times daily according to the clinical response to treatment over succeeding weeks. The results showed that 78% PHMB patients were treatment successes compared with 85.7% chlorhexidine patients (p=0.71). No serious toxic side effects occurred in any of the eyes in the study.[13] Results yielded no difference with respect to outcomes reported between PHMB and chlorhexidine. However, the sample size was small to detect clinically meaningful differences, as indicated by the wide confidence intervals of effect estimates.

A randomized, double-masked, placebo-controlled Phase 1 study was conducted to establish the ocular safety and tolerability, and systemic safety of 3 different concentrations of PHMB in healthy subjects. Safety and tolerability were compared to those of a placebo. In total 90 subjects were assigned to one of 4 treatment groups: 0.04% PHMB (n=27), 0.06% PHMB (n=27), 0.08% PHMB (n=27), or placebo (n=9). In each group, subjects received the study drug/placebo 12 times daily (1 drop every hour, daytime only) for 7 days and, if well tolerated, followed by 6 times daily (1

drop every 2 hours, daytime only) for an additional 7 days. The primary safety variable of the study was the rate of dosing limiting events (DLEs) in each treatment group. DLEs were defined as all serious adverse events (AEs) and all AEs that prompts interruption of the study drug. The purpose of the analysis was to state whether each treatment group is at significantly larger risk than placebo. During the study no SAEs occurred. Five out of the 90 subjects enrolled had a DLE (DLE rate=5.6%). The total rate of AEs was 61.1%. No DLEs occurred in the group treated with placebo and 0.04% PHMB; two DLEs occurred in the group treated with 0.06% PHMB and three DLEs in the group treated with 0.08% PHMB. For the latter group, the DLE rate was =11.1%. The events causing the withdrawn from the study were all of ocular origin and of mild to moderate intensity. The most frequent AEs reported in the study were conjunctival staining, corneal staining, pain after instillation and conjunctival hyperemia. In conclusion, only mild to moderate ocular AEs occurred after an intensive treatment with 0.08% PHMB. In addition, these AEs in patients with *Acanthamoeba* keratitis are acceptable in patients with this severe infection in whom a more concentrated solution is expected to provide a higher cure rate. For comprehensive clinical information, refer to the Investigator's Brochure for PHMB.[10]

1.1.3. Propamidine

Propamidine isethionate is an aromatic diamidine licensed in several EU countries as an antimicrobial. The antimicrobial effects of the diamidines result from the cationic surface-active properties inducing structural membrane changes affecting cell permeability. Propamidine is active against Gram-positive non-spore forming organisms, but less active against Gram-negative bacteria and spore forming organisms. It also has antifungal properties and is effective against both the trophozoite and cyst forms of *Acanthamoeba* but it is used only in combination with PHMB or chlorhexidine. Propamidine is well tolerated by ocular tissues although prolonged treatment with propamidine may lead to toxic keratopathy *in vitro* and *in vivo*.[5,14,15]

1.2. Overall Rationale for the Study

Acanthamoeba keratitis is difficult to treat. If left untreated, the condition results in blindness or corneal transplant surgery in most patients (only 1 of the first 20 patients reported before the introduction of biguanide therapy had a good outcome, 3 had medical cures of whom 1 required a corneal transplant, 3 had their eyes removed and another 14 had 1 or more therapeutic corneal transplants.[16] There are currently no drugs that are licensed for use in *Acanthamoeba* keratitis. The diamidines and biguanides are currently the most effective cysticidal anti-amoebic agents. However, the biguanides have the best amoebicidal and cysticidal activity. As a result, the current approach for the treatment for *Acanthamoeba* keratitis includes biguanides (PHMB or chlorhexidine) with or without the addition of diamidines (propamidine or hexamidine).[5] The combination of 0.02% PHMB and 0.1% propamidine is the most commonly used combination.[17] However, cases with a very poor clinical response have been reported.[5,6,13,18] Such failures are possibly related to poor drug penetration into the deep cornea.[19] The bioavailability of PHMB in the corneal stroma is likely to be increased by increasing the concentration of PHMB above 0.02%. Therefore, the use of PHMB 0.08% can be expected to reduce the treatment failures seen with PHMB 0.02%.

2. OBJECTIVES AND HYPOTHESIS

2.1. Definitions

Clinical resolution is a clinical definition, resulting from a 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 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.

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. This is the primary outcome measure. Subjects whose disease has not resolved at 12 months are considered treatment failures per protocol.

2.2. Objectives

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 with that of 0.02% PHMB + 0.1% propamidine combination therapy, 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.

A further aim of this study is to obtain additional safety information on 0.08% PHMB ophthalmic solution.

2.3. 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/SI[20]) (63% when assuming a prevalence of late stage diseases in 38% of subjects).

3. STUDY DESIGN AND RATIONALE

3.1. Overview of Study Design

3.1.1. Type of Study

This is a randomized, double-masked, double-dummy, active-controlled, multiple center, 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.

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

A total of 130 subjects affected by *Acanthamoeba* keratitis will be assigned to one of the following 2 treatment groups in a ratio of 1:1.

Group 1: 0.08% PHMB + placebo

Group 2: 0.02% PHMB + 0.1% propamidine combination therapy

Efficacy evaluations will consist of assessment of best corrected visual acuity (BCVA), pupil test (swinging light test), slit lamp examination, EuroQol five-dimension scale (EQ-5D) questionnaire and visual functioning questionnaire 25 (VFQ25).

Safety and tolerability evaluations will consist of adverse event (AE) reporting, clinical laboratory results (hematology, biochemistry and urinalysis), intraocular pressure (IOP), ophthalmoscopy, and photography of affected cornea.

3.1.2. Screening Visit

Subjects will report to the research center for the eligibility screening according to the inclusion and exclusion criteria defined in Section 4.1 and 4.2, respectively, on the day of the first drug administration (Day 0).

Subjects will receive an explanation of the purpose and nature of the study, and will be asked to review and voluntarily sign the study specific Informed Consent Form (ICF) prior to any study specific screening procedures being performed. The written informed consent will be obtained for all subjects, regardless of their eligibility for the study; the signed ICFs will be retained and archived and another copy will be provided to the subject.

For subjects who have not reached the age of majority (i.e., 18 years of age in most jurisdictions), written informed consent will also be obtained from the subject's parent(s), legal guardian(s), or legally authorized representative (LAR) (hereafter referred to as parent/guardian/LAR) prior to any study specific screening procedures being performed. The parent/guardian/LAR must also be willing to provide safety and efficacy information about the subject and oversee the administration of the investigational product. The parent/guardian/LAR must commit to accompanying the patient to each study visit.

Eligibility screening will consist of assessments as presented in the schedule of assessments (page 22).

A schematic overview of the screening procedure is presented in Figure 1.

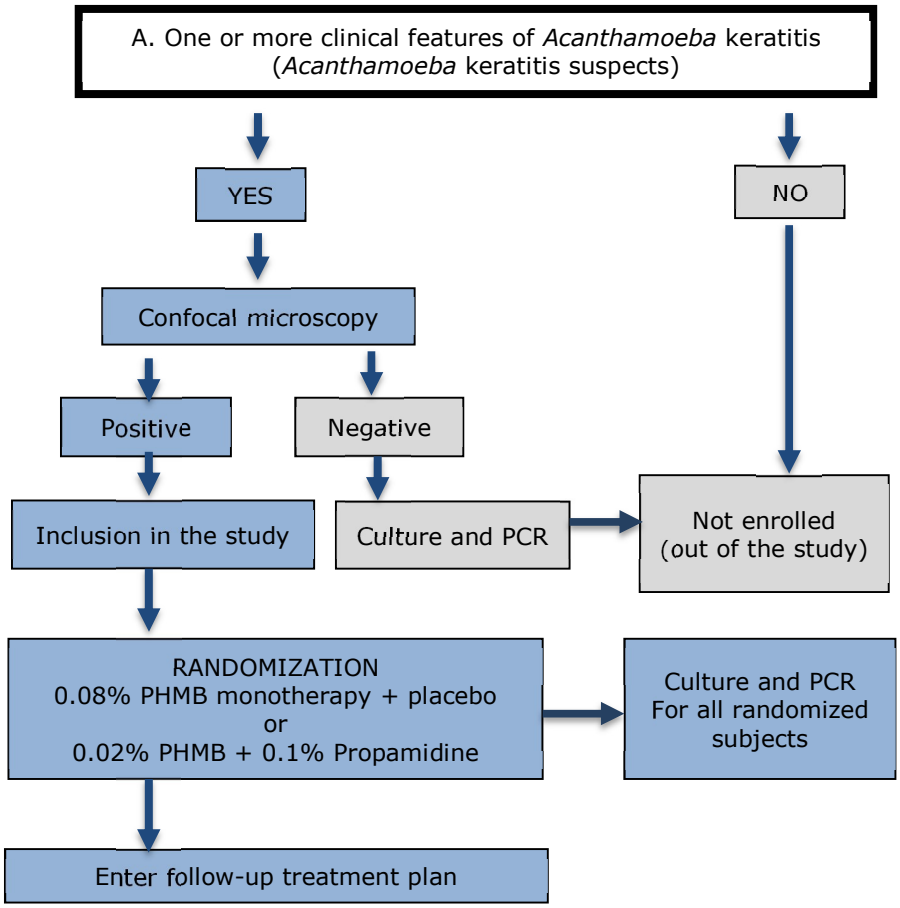


Figure 1: Schematic Presentation of the Screening Process

All subjects with clinical signs consistent with *Acanthamoeba* keratitis **and** positive confocal findings consistent with *Acanthamoeba* keratitis will be eligible for randomization providing they have not been using a topical biguanide or diamidine (see Sections 4.1 and Synopsis).

Protocol for bilateral disease: if both eyes are affected, only one eye will be treated with the study treatment and will be considered for the study. This will be the right eye, unless the severity of the infection differs in both eyes, in which case the worst affected eye should be selected. The fellow eye should be treated with the best treatment according to the clinical practice.

All subjects at screening meeting the inclusion criteria **and** including those with negative confocal microscopy findings will have corneal scrapes for culture and histology of *Acanthamoeba*, and to perform *Acanthamoeba* deoxyribonucleic acid (DNA) identification by polymerase chain reaction (PCR).

Note: this is being done for study quality, for correlation with confocal microscopy findings, and to identify patients with fungal and viral infection. Because culturing and PCR will take 2 to 8 days, during which time subjects thought clinically to have *Acanthamoeba* keratitis would be starting treatment before randomization, this assessment is not part of the inclusion criteria. Subjects who have been proven to have fungal and viral infections, if randomized on the basis of clinical and confocal microscope findings, will be removed from the study, even though they may have concomitant *Acanthamoeba* keratitis.

Subjects with a negative confocal microscopy evaluation, but a positive PCR/Culture (confocal false negative) will **not** be enrolled in the study but will be treated with the best clinically available treatment outside the study.

Subjects meeting the inclusion criteria, including findings on slit lamp examination and confocal microscopy consistent with Acanthamoeba keratitis are entered into the study and randomized to one of the two treatment arms. They will then enter the Treatment Plan and Follow-up protocol as presented in figure 2: Schematic Presentation of the Treatment and Follow-up Protocol as presented in 5.3.

3.1.3. Treatment Period

On Day 0, subjects will arrive at the clinical research center for the baseline assessments and will receive instructions on how to apply the eye drops. Subjects will be randomized to 1 of the 2 treatment groups.

Subjects will receive multiple doses of 0.08% PHMB + placebo or 0.02% PHMB + 0.1% propamidine combination therapy in the affected eye until resolution according to the treatment schedule presented in Section 5.2 Drug dosage and Administration.

On Day 0, the first application of study medication will be done at the research center after completion of all baseline assessments and randomization. Thereafter, subjects will leave the clinical research center and study drug will be self-administered at home. Subjects will return to the clinical research center for ambulant visits on Days 7, 14, 21, 30 and every 30 days until resolution.

Assessments will be performed during the treatment period as presented in the schedule of assessments.

Follow-up medical examinations will be performed 30 and 90 days after end of treatment. The specific procedures that will be performed are presented in the schedule of assessments.

3.2. Study Design Rationale

The active control for this study is combination therapy of 0.02% PHMB and 0.1% propamidine, which is the most common therapy used for treatment of patients affected by *Acanthamoeba* keratitis.[5,17] Randomization will be used to minimize bias in the assignment of subjects to treatment groups, to increase the likelihood that known and unknown subject attributes (e.g., demographic and baseline characteristics) are evenly balanced across treatment groups, and to enhance the

validity of comparisons across treatment groups. The study is double-masked to reduce potential bias during data collection and evaluation of endpoints. Since each subject will receive clinical supplies that cannot be made identical (single-use vials and multi-use containers), the study is also double dummy. To maintain the masking, subjects in each arm will use single-use vials (0.08% PHMB or 0.02% PHMB) and multi-use containers (placebo or 0.1% propamidine).

4. SUBJECT 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.

4.1. Inclusion Criteria

Each potential subject must satisfy all of the following criteria to be enrolled in the study:

1. Subject must be able and willing to give informed consent.

2. Subject must be a man or woman of any race and ≥12 years of age, inclusive. Subjects <18 years will only be enrolled in selected study sites.

3. Subject must be able to understand and willing to comply with study procedures, restrictions and requirements, as judged by the investigator.

4. Clinical findings consistent with *Acanthamoeba* keratitis.

   Clinical findings include the following:

   - Epithelial lesions: epithelial punctate keratopathy, epithelial infiltrates, epithelial defects, dendritiform epithelial ulcers.
   - Extracorneal lesions: limbal inflammation (limbitis), anterior scleral inflammation, diffuse or nodular.
   - Stromal lesions: perineural infiltrates, anterior stromal infiltrates, disciform corneal swelling, stromal ulceration, ring abscess.
   - Anterior chamber lesions: keratic precipitates, hypopyon.
   - Late findings: fixed dilated pupil, mature cataract.

5. Confocal microscopy findings consistent with *Acanthamoeba* keratitis (performed within 7 days prior to study entry or as part of screening procedures)

   - Confocal microscopy findings include: cysts are round or ovoid, may show a double wall and are 15-30 μm in size.

6. Subjects using the following previous treatments for Acanthamoeba keratitis are eligible for the study:

- **Antibiotics:** subjects who have an ocular bacterial infection at baseline are eligible for the study. However, only topical moxifloxacin is permitted, unless resistant or contraindicated.

  Note: subjects who develop intercurrent bacterial infections will be retained in the study and treated with topical moxifloxacin.

- **Antiviral drugs and antifungal drugs:** subjects are often misdiagnosed as having these infections when they have *Acanthamoeba* keratitis. Subjects taking antivirals and antifungal agents (except for any using PHMB or Chlorhexidine) for a misdiagnosis can be included, but must discontinue these drugs after entry into the study.

  Note: subjects who are thought to have combined *Acanthamoeba* keratitis with herpes or fungal keratitis are excluded from the study.

- **Anti-inflammatory drugs:** Subjects using topical steroids and/or oral NSAIDs before the diagnosis of *Acanthamoeba* keratitis are eligible for the study. However, these subjects must agree to change therapy to the topical steroids and oral NSAIDs that are specified for use in the study (as described in section 4.3 Concomitant Medication and Other Restrictions during Study).

7. Females of childbearing potential will be included if they are either sexually inactive (sexually abstinent for 14 days prior to the first study drug dose continuing through 28 days after the last study drug dose, or using one of the following highly effective contraceptive (i.e. results in <1% failure rate when used consistently and correctly) methods in this study:

   a. intrauterine device (IUD);

   b. surgical sterilization of the partner (vasectomy for 6 months minimum);

   c. combined (estrogen or progestogen containing) hormonal contraception associated with the inhibition of ovulation (either oral, intravaginal, or transdermal);

   d. progestogen only hormonal contraception associated with the inhibition of ovulation (either oral, injectable, or implantable);

   e. intrauterine hormone releasing system (IUS);

   f. bilateral tubal occlusion.

   Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. In this study, abstinence is only acceptable if in line with the subjects preferred and usual lifestyle.

   Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM) are not

acceptable methods of contraception. As well, a female condom and a male condom should not be used together.

8. Females of childbearing potential agree to remain sexually inactive or to keep the same birth control method for at least 28 days following the last study drug dose.

9. A female of non-childbearing potential must have undergone one of the following sterilization procedures at least 6 months prior to the first study drug dose:

   a. hysteroscopic sterilization;

   b. bilateral tubal ligation or bilateral salpingectomy;

   c. hysterectomy;

   d. bilateral oophorectomy;

   or be postmenopausal with amenorrhea for at least 1 year prior to the first study drug dose and follicle stimulating hormone (FSH) serum levels consistent with postmenopausal status

10. A non-vasectomized male subject agrees to use a condom with spermicide or abstain from sexual intercourse during the study until 90 days beyond the last dose of study drug and the female partner agrees to comply with inclusion 7 or 8. For a vasectomized male who has had his vasectomy 6 months or more prior to study start, it is required that they use a condom during sexual intercourse. A male who has been vasectomized less than 6 months prior to study start must follow the same restrictions as a non-vasectomized male.

    Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and LAM are not acceptable methods of contraception. As well, a female condom and a male condom should not be used together.

11. If male, they must agree not to donate sperm from the first study drug dose until 90 days after dosing.

4.2. Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from participation in the study:

1. Subject with documented history and/or clinical signs of concomitant presence of an ocular infection caused by viruses (herpes simplex virus [HSV]) or fungi.

2. Subject treated with drugs having effects on *Acanthamoeba* cysts prior to study entry, including biguanides (PHMB, chlorhexidine) and diamidines (propamidine, hexamidine).

3. Subjects requiring systemic immunosuppression for *Acanthamoeba* associated scleritis.

4. Subjects requiring urgent surgical intervention for advanced *Acanthamoeba* keratitis in either eye (e.g., for advanced corneal thinning/melting etc.).

5. Subject with known or suspected allergy to biguanides, diamidines or intolerance to any other ingredient of the investigational treatments.

6. Subject affected by immunodeficiency diseases or taking systemic immunosuppressive therapy.

7. Subject with a major systemic disease or other illness that would, in the opinion of the investigator, compromise subject's safety or interfere with the collection or interpretation of study results.

8. If female, pregnancy, planned pregnancy, or breast-feeding.

9. Subject is participating in another interventional clinical study with an experimental or unapproved/unlicensed therapy or has participated in another interventional clinical study within 4 weeks prior to this study.

The investigator must ensure that all study enrolment criteria have been met at randomization. If a subject's status changes after randomization, but before the first dose of study drug is given such that he or she no longer meets all eligibility criteria, then the subject should be excluded from participation in the study.

4.3. Concomitant Medication and Other Restrictions during Study

Potential subjects must be willing and able to adhere to the following prohibitions and restrictions during the course of the study to be eligible for participation:

1. Female subjects of childbearing potential have to be sexually inactive (abstinent) or have to use an acceptable birth control method as described in Section 4.1 during the study and continuing through 28 days after the last dose of study medication.

2. Non-vasectomized male subjects have to use an acceptable birth control method as described in Section 4.1 during the study continuing through 90 days after the last dose of study medication. Female partners should also use an acceptable birth control method as described in Section 4.1 during the study and continuing through 28 days after the last dose of study medication.

3. Subjects are allowed to use the following concomitant medications during the study:

   Antibiotics: Topical moxifloxacin is permitted for the treatment of intercurrent bacterial infections (unless culture and sensitivity, or clinical progress demands a change). Topical moxifloxacin is not permitted for use as a prophylactic antibiotic in patients with corneal ulcers; PHMB is a good broad spectrum anti-bacterial and an additional antibacterial is not needed for this. The value of prophylaxis is unproven.

   Antiviral drugs and antifungal drugs: The use of these drugs is *not* allowed during the study. If subjects are using antiviral or antifungal drugs at study entry, they must be discontinued.

Anti-inflammatory drugs: For subjects on steroids at study entry (already using topical steroids for e.g. mistaken diagnosis of HSV keratitis or as adjunctive treatment for bacterial keratitis), there are the following options:

a. Either stop steroids, OR maintain, OR reduce the doses (at the investigator's discretion). Unpreserved Dexamethasone (0.1% or 0.15%) is the only topical steroid permitted for use in this trial. Patients using any other topical steroids on trial entry should be changed to this at the appropriate frequency. Diclofenac is the only oral NSAID permitted in the trial and will be ADDED at the appropriate dose (75 mg to 150 mg daily, divided in two or three doses) and continued at any level while topical steroids are in use during the study.

b. Subjects using topical NSAIDs and ciclosporin at study entry should have these discontinued after randomization.

c. Subjects using no topical steroids at study entry can have these started together with oral NSAIDs (recommended diclofenac, 75 mg to 150 mg daily, divided in two or three doses) during the study as specified in the schematic overview of the Treatment and Follow-up Protocol.

    i. Dosing frequency of topical dexamethasone (0.1% or 0.15%) : at the discretion of the principal investigator between 1 drop every second day to hourly depending on the degree of inflammation.

    ii. Dose of diclofenac: at the discretion of the principal investigator. The recommended dosing is 75 mg to 150 mg daily, divided in two or three doses depending on the degree of inflammation

Other permitted topical mediations: Unpreserved lubricants, mydriatics (cyclopentolate, homatropine or atropine) and glaucoma medications are permitted. For glaucoma medications a timolol preparation is recommended as first line (for its low toxicity) and prostaglandin analogues as last resort because of their potential to increase inflammation.

All concomitant medication used in relation to the current eye infection will be reported. For the remaining concomitant medication, only medication in use within 30 days before signing the ICF will be reported in the case report form (CRF). The name of the drug, start and stop date, the indication for use, the total daily dose and route of administration will be recorded. Medications will be coded by standardized dictionary.

4.4. Subject Completion/Withdrawal

Any subject may voluntarily discontinue the study at any time without prejudice. The investigator may elect to discontinue a subject for reasons unrelated to the study drug (e.g. failure to comply with the study protocol, missed visits etc.), for reasons related to the study drug ([serious] adverse event ([S]AE])) or in case of treatment failure. In either event, reason(s) for discontinuation should be recorded on the CRF. Possible reasons for study discontinuation include the following:

– Ocular intolerance.

- (S)AEs necessitating discontinuation from the study.
- Treatment failure.
- The subject is lost to follow-up.
- Subject decision not related to an (S)AE.
- Investigator decision (specify).
- Other reason (specify).

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.

If the subject discontinues, all the end-of-study visit assessments must be performed, if possible. Moreover, the investigator should ensure (as far as possible) that all used and unused study drug has been collected from the subject. The subject will exit the study and be treated at the investigator's discretion. Discontinued subjects will not be replaced.

Subjects discontinued for (S)AE(s) will be followed-up after subject's discontinuation until the event is resolved or considered medically stable by the investigator. If necessary, the investigator may schedule further visits at his/her discretion. Data collected in the period up to the end-of-study visit will be recorded in the CRF. Data collected after Day 90 following the end of treatment, and related to the (S)AE that led to discontinuation will be recorded in the subject's medical record, and in the sponsor's Safety Database.

In case a subject is lost-to-follow-up, the investigator will attempt to contact the subject (by phone, letter or e-mail) at least twice.

A subject will be considered to have completed the study if all required assessments up to and including the 90 days after end of treatment and end-of-study assessments have been completed.

5. TREATMENTS

5.1. Treatment Assignment

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

Protocol for bilateral disease: if both eyes are affected, only one eye will be treated with the study treatment and will be considered for the study. This will be the right eye, unless the severity of the infection differs in both eyes, in which case the worst affected eye should be selected. The fellow eye should be treated with the best treatment according to the clinical practice.

Subjects will be assigned to the following treatment groups:

Group 1: 0.08% PHMB + placebo

Group 2: 0.02% PHMB + 0.1% propamidine combination therapy

5.2. Drug dosage and Administration

On Day 0, subjects will receive instructions on how to apply the eye drops. Subjects will receive the assigned treatment to be administered in the affected eye until resolution. Subjects will be treated for a maximum of 1 year after randomization and subjects meeting the criteria for clinical resolution at or before 12 months will be followed up for 90 days before completing the study.

Subjects will receive an initial intensive course of 0.08% PHMB + placebo or 0.02% PHMB + 0.1% propamidine combination therapy.

Treatment throughout the study will be given using 1 drop of each ophthalmic solution in the study eye at different frequencies during the daytime only. At study entry, an intensive 19-day antiamoebic treatment protocol will be initiated as follows:

Day 0 to 5*	1-hourly drops (16 drops in a day) for 5 days
Day 6 to 12	2-hourly drops (8 drops in a day) for 7 days
Day 13 to 19	3-hourly drops (6 drops in a day) for 7 days
Day 20	Then 4x daily thereafter

* on the first day of treatment (after randomization) 16 drops might not be feasible, in this case the treatment should be applied as many times as possible but not exceeding the hourly regimen.

Notes on treatment

i. The frequency will be maintained at 4x daily unless there is a relapse (see Box XI, Relapse on page 42). In the event of a relapse, culture negative subjects will receive the same intensive 19-day treatment course as given at study entry.

ii. Antiamoebic drops will be discontinued when clinical resolution is reached (see Figure 2 and Legend IV Discontinue treatment).

iii. Clinical resolution is a clinical definition, resulting from a 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 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.

For subjects on steroids or oral NSAIDs at study entry (already using topical steroids for e.g. mistaken diagnosis of HSV keratitis or as adjunctive treatment for bacterial keratitis), the same intensive 19-day antiamoebic treatment protocol will be used. For their steroid use there are the following options:

- Either stop steroids, **OR** maintain, **OR** reduce the doses (at the investigator's discretion). Unpreserved Dexamethasone (0.1% or 0.15%) is the only topical steroid permitted for use in this trial. Patients using any other topical steroids on trial entry should be changed to this at the appropriate frequency. Diclofenac is the only oral NSAID permitted in the trial and will be ADDED at the appropriate dose (usually 75 mg to 150 mg three times daily) and continued at any level while topical steroids are in use during the study.

Detailed instructions about the use of concomitant medication and adjunctive therapy are given in Section 4.3 "Concomitant medication and other restrictions during study" and also in Section 5.3 "Treatment and Follow up Protocol"
The study drug(s) will be applied to the affected eye. In case both eyes are affected, only 1 eye will be treated with study treatment. This will be the right eye, unless the severity of the infection differs between eyes, in which case the worst affected eye should be selected. The fellow eye should be treated with the best treatment according to the clinical practice.

One drop of 0.1% propamidine or placebo (multiple-use containers) will be administered first, followed after a minimum of 5 minutes by 0.08% or 0.02% PHMB (single-use vials).

The single-use vials should be used immediately after opening and the remaining solution should be discarded. The multiple-use containers should be closed after each use and kept for up to 28 days after opening.

On Day 0, study drugs will be administered after completion of all baseline assessments and randomization. The first application will be done at the clinical research center by the subject under supervision of a member of the study team. Thereafter, study drugs will be applied at home.

Before administration, both single-use vials and the multiple-use containers should be shaken.

If the subject misses a dose of study drug, the subject should wait and take the next dose according to the treatment schedule. 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 following the start of treatment should not be discontinued, but will be classified as non-compliant. These subjects will be included in the Full Analysis Set and excluded from the analysis of the Per-Protocol Analysis Set (see Section 7.17.1).

The vials and containers should be stored at a temperature lower than 25°C (or at room temperature) and protected from freezing. Research center staff will instruct subjects on how to store study drug for at-home use.

5.3. Treatment and Follow-up Protocol

A schematic overview of the treatment and follow-up period is presented in figure 2.

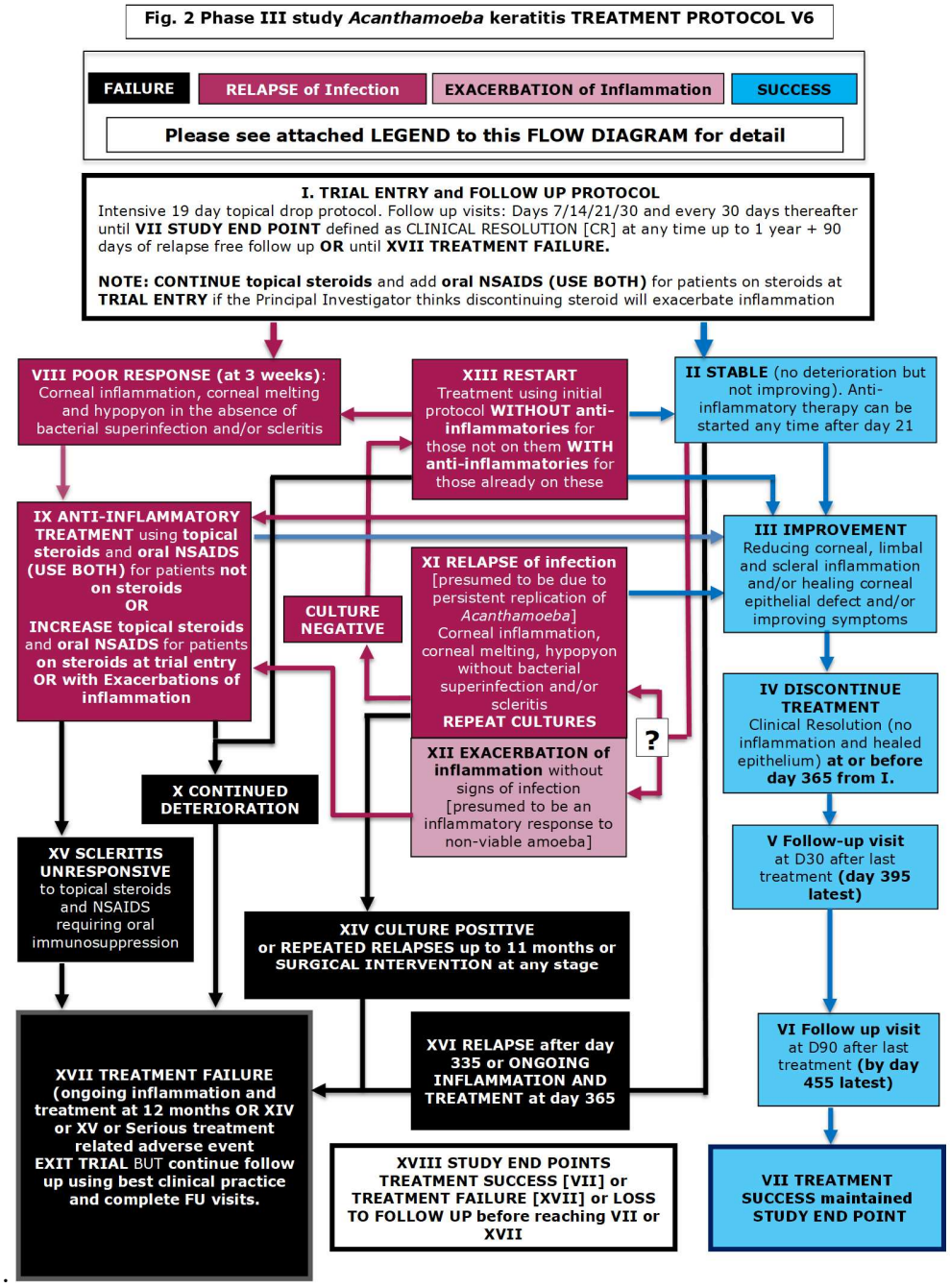


Figure 2: Schematic Presentation of the Treatment and Follow-up Protocol

Legend for Figure 2 - The text boxes and lines in the figure are color coded:

- **Pale blue text boxes and lines** for responding disease or treatment success and **STUDY END POINT**
- **Red text boxes and lines** for poorly responsive and recurrent / relapsing disease presumed to be due to replication of *Acanthamoeba*
- **Pink text box for** exacerbations of inflammation presumed to be due to flare ups of inflammation secondary to the immune response to non-viable *Acanthamoeba*
- Black text boxes for treatment failure endpoints ALL leading to TRIAL EXIT

This protocol defines the treatment of patients. Treatment options are defined as far as possible to reduce variations, which will complicate or invalidate the analysis of the results, whilst maintaining optimal standards of care based on current evidence and expert opinion. The changes of therapy in this protocol, and therapeutic failures, are determined by both the development and resolution of clinical signs of inflammation and ulceration, as well as the identification of *Acanthamoeba* by culture during disease relapses.

Each box is labelled with a Roman numeral I to XVIII. The legend below describes the rationale for each step:

BOX I. FOLLOW UP PROTOCOL from day zero at TRIAL ENTRY.

Treatment throughout the study is given using 1 drop of each ophthalmic solution in the study eye at different frequencies during the daytime only.

At study entry initiate an intensive 19-day antiamoebic treatment protocol:

Day 0 to 5	1-hourly drops (16 drops in a day) for 5 days
Day 6 to 12	2-hourly drops (8 drops in a day) for 7 days
Day 13 to 19	3-hourly drops (6 drops in a day) for 7 days
Day 20	then 4x daily thereafter

a. This frequency is maintained at 4x daily unless a relapse (BOX XI - RELAPSE) occurs. In case of a relapse, culture negative subjects receive the same intensive 19-day treatment course as given at study entry.

b. Antiamoebic drops are discontinued when IV - DISCONTINUE TREATMENT is reached.

In subjects on steroids at TRIAL ENTRY: (already using topical steroids for e.g. mistaken diagnosis of HSV keratitis or as adjunctive treatment for bacterial keratitis), the same intensive 19-day antiamoebic treatment protocol is used. For their steroid use there are these options:

a. Either stop steroids, **OR** maintain steroids, **OR** reduce the doses of steroids (at the investigator's discretion). Change steroid therapy to unpreserved topical dexamethasone (0.1% or 0.15%) if not using this already. When steroid is to be continued at any level; ADD an oral non-steroidal anti-inflammatory drug

(NSAID). The oral NSAID used in the trial is diclofenac; change therapy to diclofenac if patient is on alternative NSAID at study entry.

Dexamethasone (0.1% or 0.15%) drops and oral diclofenac are permitted in the study and may be discontinued, tapered or continued on inclusion into the study at the discretion of the investigator:

- Dosing frequency of topical dexamethasone (0.1% or 0.15%): between 1 drop every second day to hourly depending on the degree of inflammation.
- Dose of diclofenac: The recommended dosing is 75 mg to 150 mg daily, divided in two or three doses depending on the degree of inflammation

For subjects using antifungals or antivirals on study entry: discontinue antifungals or antivirals.

For subjects using topical antibiotics on study entry for concurrent bacterial keratitis: alter antibiotic to moxifloxacin unless there is evidence from microbiology studies that the bacteria are resistant to moxifloxacin.

Prophylactic antibiotics for patients with ulceration: discontinue prophylactic antibiotics.

Other permitted topical mediations: Unpreserved lubricants, mydriatics (cyclopentolate, homatropine or atropine) and glaucoma medications are permitted. For glaucoma medications a timolol preparation is recommended as first line (for its low toxicity) and prostaglandin analogues as last resort because of their potential to increase inflammation.

After the 20 day treatment in Box I patients will either stabilize or improve and move to the treatment pathway in Box II or deteriorate and move to the pathway in Box VIII.

5.3.1. Responding Disease or Treatment Success

BOX II STABLE

Stable at 3 weeks is defined as no deterioration but not improving. These STABLE subjects may improve or not over the next few weeks. If no improvement, then these patients may be started on anti-inflammatory treatment as in Box IX (no re-culture needed. Alternatively the inflammation may deteriorate (this may occur at any stage of treatment) and the rationale for managing this problem is described here:

DETERIORATION of inflammation (after excluding co-infection with other organisms).

The two possibilities which the PI needs to differentiate between are:

a. Relapse of infection (Box XI) presumed or proven to be due to continued replication of Acanthamoeba) OR

b. Exacerbation of inflammation (Box XII) presumed to be due to inflammation secondary to the immune response to non-viable Acanthamoeba

XI RELAPSE of infection is identified by a positive culture, unfortunately very insensitive die 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. This is as opposed to:

XII EXACERBATION of inflammation: which is accompanied by mild conjunctival or corneal inflammation (sometimes coarse anterior stromal infiltrates like those following adenovirus keratitis) and/or mild scleritis. XV SCLERITIS unresponsive to topical steroids and oral NSAIDS is another cause of this: these patients are complex to manage and as a result we decided that these should exit the trial.

Once a decision has been made between these two the appropriate protocol is followed for each scenario as outlined in XI and XII.

Note: if this occurs after 11 month (335 Days) it is a treatment failure XVII (as patients relapsing at this point will not have time to respond to treatment) and the patient exits the trial.

BOX III IMPROVEMENT

Reducing inflammation and some healing of any ulcers and/or improving symptoms. These subjects may continue to improve to the point they go to IV Discontinue treatment.
Deterioration of inflammation: Patients in this subset may deteriorate due to: XI Relapse of Infection or XII Exacerbation of inflammation in which case they are managed as described in paragraph II a, b for these issues.

BOX IV DISCONTINUE TREATMENT

DISCONTINUE TREATMENT (includes topical steroids, oral NSAIDs and topical antiamoebics/microbials) is when any ulcers have healed and all signs of *Acanthamoeba* keratitis related inflammation have resolved (see detailed description in the protocol; mild conjunctival inflammation related to drugs or other condition such as blepharitis are acceptable) at or before 12 months (365 days) from study entry. Clinical resolution (CR) after 365 days is a failure (see Box XVI - RELAPSE AFTER 11 MONTHS). It is recommended that topical steroids are discontinued 2-4 weeks before discontinuing topical anti-amoebics.

BOX V Follow-up visit (Day 30 after last treatment)

Deterioration of inflammation: Patients in this subset may deteriorate due to: XI Relapse of Infection or XII Exacerbation of inflammation in which case they are managed as described in paragraph II a, b for these issues.

BOX VI Follow-up visit (Day 90 after last treatment)

Follow-up visit 90 days (D90) after last treatment has two outcomes: if there are no signs of a relapse by 15 months (Day 455) at latest, the pathway goes to Box **VII** - TREATMENT SUCCESS maintained STUDY-END-POINT. In the event of signs of a RELAPSE, the pathway is the same as for relapses in box XI or XIIP).

BOX VII Treatment success maintained study end point. EXIT TRIAL

5.3.2. Poor Response at 3 weeks or Treatment Failure

BOX VIII POOR RESPONSE at 3 weeks.

Poor response at 3 weeks from I Trial entry or XIII RESTART: patients are managed using the guidelines in IX. Criteria for this include enlarging ulcer, stromal thinning, deteriorating corneal, inflammation (infiltrate including ring abscess), intraocular inflammation (uveitis and hypopyon) and/or scleritis.

BOX IX INTRODUCE ANTI-INFLAMMATORY TREATMENT

a. For subjects NOT using topical steroids start these WITH oral NSAIDs. The topical steroid that is permitted in the Trial is unpreserved dexamethasone (0.1% or 0.15%). The oral NSAID used in the trial is diclofenac. The frequency of the drops and the dose of oral NSAID that is used is at the discretion of the Investigator.

b. For subjects already on unpreserved topical dexamethasone (0.1% or 0.15%) and oral diclofenac, increase the frequency of the steroid drops and dosage of oral diclofenac when possible.

This is expected to result in an improvement for most cases and they move to BOX II.1 STABLE or BOX III IMPROVEMENT.

BOX X CONTINUED DETERIORATION

Alternatively, if continued deterioration occurs, cases move to Box XVIII - TREATMENT FAILURE and EXIT Trial.

5.3.3. Relapse

BOX XI **RELAPSE WITHIN 11 MONTHS**

Relapse within 11 months, after an initial response is common. Cultures for *Acanthamoeba* and other organisms (fungi, bacteria and herpes) must be repeated, deriving from a repeated corneal biopsy, if the investigator thinks this is appropriate.

c. If the CULTURES are NEGATIVE patients this may mean that the anti-amoebic therapy is having some effect although we have no way of being certain about this (confocal and PCR don't help as these can be positive when the organisms are non-viable). These patients are managed as described in XIII below with another 19 day course of intensive therapy, with or without anti-inflammatory therapy depending on status.

d. If CULTURES are POSITIVE patients have TREATMENT FAILURE and managed as described in XIV and XVII below.

Box XII. EXACERBATION of inflammation is presumed to be due to inflammation secondary to the immune response to non-viable Acanthamoeba and anti-inflammatory treatment can be re-started or increased at the discretion of the PI without the need for re-culture and a further course of intensive anti-amoebic therapy. IMPORTANT: if anti-amoebic treatment had been stopped restart this when anti-inflammatory treatment is re-started.

Distinguishing between XI and XII is often difficult and guidelines for this are given in II a, b above.

Box XIII. Restart with another 19 day intensive course of anti-amoebic therapy with or without topical steroids and oral NSAIDS:

a. If patients were not on these at the time of the relapse of infection we recommend not using these until after the 19 day course of intensive treatment and depending on response move to as above.

b. If they were on them at the time of the relapse continue at a dose determined by the PI and follow pathways II, III, IX or X.

Box XIV. CULTURE POSITIVE these patients are Exit Trial and are managed as described in XVII below.

Box XV. SCLERITIS UNRESPONSIVE to treatment in IX. This is a failure (see XVII below).

Box XVI. RELAPSE after day 335 or ingoing inflammation and treatment at day 365: this is a treatment failure and is managed as described in XVII below

Box XVII. TREATMENT FAILURE and EXIT TRIAL. The treatment randomization allocation will be opened by the Sponsor at this point. Trial drug (PHMB 0.08%) will be available to the patient, if requested by the Investigator, providing the patient continues trial follow up for safety evaluation.

XVIII. Summary of Study end points.

5.3.4. End of Study

XIX. Summary of Study end points.

End-of-study is defined as the last follow-up visit at 90 days after end of treatment, or the day of study discontinuation. Discontinuation because of clinical failure is defined as follows:

1. Clinical resolution not achieved after 1 year of study treatment
2. Clinical relapse within 3 months after end-of-treatment, if occurring more than 12 months after randomization

5.4. Identity of Investigational Product

Test treatment: 0.08% PHMB + placebo

Name	: *PHMB*
Active compound	: polyhexamethylene biguanide
Activity	: Cationic surface-active antimicrobial agents
Indication	: Not applicable
Strength	: 0.08%
Dosage form	: eye-drops solution provided in 0.3 mL single-use vials
Manufacturer	: SIFI SpA, Italy

Name	: *placebo*
Substance	: sodium chloride, sodium phosphate buffer, benzalkonium chloride and water for injections
Active compound	: not applicable
Dosage form	: eye-drops solution provided in 10 mL multiple-use containers, visually identical to test reference treatment (propamidine multiple-use containers)
Manufacturer	: SIFI SpA, Italy

Reference treatment: 0.02% PHMB + 0.1% propamidine combination therapy

Name	: *PHMB*
Active compound	: polyhexamethylene biguanide
Activity	: Cationic surface-active antimicrobial agents
Indication	: not applicable
Strength	: 0.02%
Dosage form	: eye-drops solution provided in 0.3 mL single-use vials
Manufacturer	: SIFI SpA, Italy

Name	: *propamidine (Brolene)*
Active compound	: propamidine isethionate
Activity	: bacteriostatic properties against a wide range of organisms
Indication	: minor eye infections
Strength	: 0.1%
Dosage form	: eye-drops solution provided in 10 mL multiple-use containers
Manufacturer	: Sanofi, United Kingdom

Excipients of PHMB are sodium chloride, sodium phosphate buffer and water for injections.

Excipients of propamidine are ammonium chloride, sodium chloride, benzalkonium chloride, sodium hydroxide, water for injections.

Excipients of placebo are sodium chloride, sodium phosphate buffer, benzalkonium chloride and water for injections.

5.5. Selection of Doses in the Study

There is consensus that a combination of 0.02% PHMB and 0.1% propamidine eye drops is effective for treatment of *Acanthamoeba* keratitis. However, cases with very poor clinical response have been reported. These failures are possibly related to poor drug penetration into the deep cornea. Therefore, the bioavailability of PHMB in the corneal stroma is likely to be increased by increasing the concentration of PHMB above 0.02%. A randomized, double-masked, placebo-controlled Phase 1 study was conducted to establish the ocular safety and tolerability, and systemic safety of 3 different concentrations of PHMB in healthy subjects (0.04%, 0.06% and 0.08% administered 12 times daily (1 drop every hour, daytime only) for 7 days followed by 6 times daily (1 drop every 2 hours, daytime only) for an additional 7 days). This study showed that intensive administration of 0.08% PHMB is safe and well tolerated in healthy subjects. Therefore, consistent with the CHMP advice from the EMA provided in July 2014, the highest tolerated concentration tested in the Phase 1 study (0.08%) will be used in this Phase III study in subjects with *Acanthamoeba* keratitis.

5.6. Masking

This study is designed as double-masked, double-dummy study. However, the bottle of the dummy (placebo), although very similar, is not identical to the bottle of the study product, as it was not possible to obtain an identical bottle. Because unmasking is allowed per protocol in case of lack of efficacy, there is a small risk of disclosing of the treatment assignment for subjects continuing to participate in the study. To reduce this risk, and to protect the integrity of the data, efficacy assessments should be performed by study personnel not having access to the study treatment, dispensing logs, accountability forms, the eCRF, or other sources of treatment assignment information (assessor-masked design). In particular the records of unmasked subjects should not be seen by the doctors carrying out the study. The investigator and other study staff, the subjects, the monitors and the sponsor will only have access to masked treatment assignment information on a ‘need to know basis’ until data collection has been completed, the database is locked and the protocol deviations and the primary reason for discontinuation from the study for each subject are determined. The exact requirements and process will be outlined in the Study Operations Manual.

The randomization code does not disclose any treatment information. Until the moment of unmasking, the treatment assignments linked to the codes are accessible only by the individuals appointed by the Sponsor as Qualified Persons responsible for Pharmacovigilance. Emergency Unmasking should only be done if knowledge of treatment assignment is considered relevant for medical care of the patient. Unmasking is an Investigator’s responsibility and, if in doubt, can be discussed with the Medical Monitor. Details will be provided in the Study Operations Manual.

Subjects will be treated for a maximum of 1 year after randomization. Study treatment may be unmasked for serious safety reasons. These unmasked subjects will be discontinued from the study

The randomization code will be broken when all subjects have finished the study.

5.7. Drug Accountability

The investigational site will maintain an inventory of study drug supplied by the Sponsor. All drug supplies must be kept in a locked room that can be accessed only by the pharmacist, the investigator, or another duly designated person. Specifically study staff performing efficacy assessments must not have access to the study drug. This study drug must not be used outside the context of this protocol. Under no circumstances should the investigator or site personnel supply study drug to other investigators or clinics, or allow the supplies to be used other than as directed by this protocol without prior authorization from the sponsor.

Adequate records on receipt, use, return, loss, or other disposition of medication must be maintained. A specific drug accountability form or computer records used by the pharmacy at the investigational center, can be used to provide drug accountability information. In either case, information describing study drug supplies and the dispensing record, subject by subject, must be provided, signed by the investigator (or the pharmacist or other person who dispensed the drug) and collected by the local study monitor. Requisite data includes relevant dates, quantities, batches or code numbers, and subject identification for subjects who received study drug.

The investigator or designated study-site personnel will maintain a log of all study drug dispensed and returned. Drug supplies for each subject will be inventoried and accounted for throughout the study. When study drug is self-administered by subjects, the number of single-use vials and multiple-use containers with study drug dispensed will be recorded and compared with the number of used and unused vials returned.

At the end of the study, following authorization by study management and on agreement of the sponsor, drug may be destroyed on site as dictated by the appropriate standard procedures at the participating institution. Destruction must be documented. Alternatively, all unused products will be collected by the local monitor and returned for destruction.

6. STUDY EVALUATIONS

6.1. Study Procedures

In the case of both eyes being affected, all study procedures will be performed in the ‘study eye’ only.

6.1.1. Overview

The schedule of assessments, presented on page 22, summarizes the frequency and timing of assessments applicable to this study.

6.1.2. Screening Measurements

Identification of *Acanthamoeba* by Confocal Microscopy

The subject will undergo confocal microscopy before any corneal scrapings. Confocal microscopy will be performed according to Standard Operating Procedures and applying a standard set of evaluation criteria, the Investigator is responsible for complying to this. Details will be provided in the Study Operations Manual. For each subject, 1 full-thickness scan that include endothelial, stromal, and epithelial images will be taken of the central cornea, regardless of infiltrate location. In addition, at least 1 additional full-thickness scan will be taken in the area of the infiltrate, by instructing the subject to look in the appropriate direction. Readers will also comment on the presence or absence of trophozoite-like and cyst-like structures (presumed to be *Acanthamoeba*), perineural inflammation, filaments or hyphae (presumed to be fungus), and inflammatory infiltrate, as well as the anatomic location of each of these findings (endothelial, posterior stroma, anterior stroma, or epithelial). Cysts will be defined as high contrast, hyper-reflective, spherical structures measuring 15 to 30 µm. Graders will record whether any cysts are present in the group of images. A positive confocal test will have at least 1 cyst.

Images will be sent to a central reading center, however, the investigator's decision will be accepted for inclusion in the study.

6.1.3. Measurements for Routine Clinical Management

Identification of *Acanthamoeba* by microbiological culture or cytological smear

Corneal scrapings will be obtained under topical anesthesia using an aseptic technique.

Cytopathology techniques
To ensure homogeneity smears should be examined using preferably calcafluor white stain at all centers. Laboratories may use any additional techniques according to their local protocols.

Culture techniques
The study specimen (corneal epithelium, a corneal ulcer scrape or a corneal biopsy) should be cultured on non-nutrient agar plates inoculated with E. coli and processed according to standard local routine microbiological procedures. Any changes in culture techniques during the course of the study should be reported.

Identification of *Acanthamoeba* DNA by PCR

PCR will be performed locally according to standard local routine procedures.

6.1.4. Baseline Measurements

Vital Signs

Blood pressure, pulse rate and body temperature will be assessed with the subject in supine position after 5 minutes of rest.

6.1.5. Efficacy Measurements

All efficacy measurements should be performed by a staff member not having access to treatment assignment.

Efficacy evaluations will consist of assessment of BCVA, pupil test (swinging light test), slit lamp examination, EQ-5D questionnaire and VFQ25 questionnaire. Assessments will be performed in accordance with the schedule of assessments. When multiple assessments are scheduled at the same time, completion of questionnaires by the subject will be done before ocular assessments to be performed by the investigator.

6.1.5.1. Ocular Assessments by Investigator

Best corrected visual acuity (BCVA)

BCVA will be 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.

Pupil test (swinging light test)

A pupil test (swinging light test also known as a test for a relative afferent pupillary defect) will be used to detect retinal or optic nerve involvement when the retina cannot be examined due to the presence of corneal opacity or cataract. Detailed information is in the Study Operation Manual.

Slit lamp examination

Slit lamp biomicroscopy will be performed by the examining ophthalmologist, using a slit lamp biomicroscope. Detailed information about the procedure is provided in the Study Operation Manual.

6.1.5.2. Subject-reported outcomes

Subjects will complete quality of life questionnaires. If the subject is unable to complete the questionnaires without assistance, the questionnaires may be verbally answered and the subject's responses recorded by site personnel or a chaperone.

EQ-5D

The EQ-5D questionnaire will be completed by all subjects. Subjects being ≤15 years of age will complete the youth version of this questionnaire. The EQ-5D questionnaire is a standardized self-administered instrument for use as a measure of health outcome. An example of the questionnaire is provided in Appendix 3.

EQ-5D questionnaire scores will be calculated according to the standard procedure of the questionnaire.

Visual Functioning Questionnaire (VFQ25)

The VFQ25 questionnaire will be completed, in addition to the EQ-5D, by all subjects over 18 years of age. An example of the questionnaire is provided in Appendix 3. VFQ25 questionnaire scores will be calculated according to the standard procedure of the questionnaire.

6.1.6. Safety and Tolerability Measurements

Safety and tolerability evaluations will consist of AE reporting, clinical laboratory (hematology, biochemistry and urinalysis), IOP, ophthalmoscopy, and photography of affected cornea. Assessments will be performed in accordance with the schedule of assessments. Between clinical research centers, assessments will be standardized as much as possible.

Adverse Events

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 0, Adverse Event Reporting.

Clinical Laboratory Tests

Blood samples for serum chemistry and hematology and a mid-stream urine sample for urinalysis will be collected at the time points indicated in the schedule of assessments. The local clinical laboratory will clearly mark all laboratory test values that are outside the normal range and the investigator will indicate which of these deviations are clinically significant or not. The investigator must record all clinically significant abnormal values during the study in the AE section of the CRF.

Clinical significance is defined as any variation in signs, symptoms, or testing that has medical relevance according to the investigator and may result in an alteration in medical care.

The following tests will be performed:

- Hematology: full blood count (total red blood cell count / white blood cell count with differential), hematocrit, hemoglobin, platelets.

- Blood biochemistry: serum creatinine, aspartate aminotransferase, alanine aminotransferase, glucose, serum electrolytes (sodium, potassium), total protein, total bilirubin, alkaline phosphatase, gamma-glutamyl transferase, cholesterol and triglycerides.
- Urinalysis (by dipstick): specific gravity, pH, protein, glucose, ketones, urobilinogen, blood (erythrocytes, leucocytes).
- Pregnancy testing in urine (by dipstick) (for women of childbearing potential only).

Intraocular Pressure

At each visit, IOP measurement will be done using any CE marked tonometer (e.g., Goldmann, Icare or non-contact tonometers). For consistency, subjects should preferably be tested using the same equipment at each visit.

Ophthalmoscopy

Ophthalmoscopy will be performed after pupil dilatation to examine the vitreous, retina, macula and choroid at screening and at the final visit AND at any time if the vision deteriorates or either the pupil tests or IOP are abnormal.

6.2. Appropriateness of Measurements

The assessments which will be performed in this study are standard, and generally recognized as reliable, accurate and relevant.

6.2.1. Timing of Assessments

The date of all assessments will be recorded in the CRF.

When multiple assessments are scheduled at the same time, completion of questionnaires by the subject will be done before ocular assessments to be performed by the investigator.

6.3. Primary Efficacy Variables

The primary efficacy variable chosen to assess drug efficacy is the CRR_12 (the clinical resolution rate at 12 months from randomization, 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 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.

6.4. Secondary Variables

The secondary efficacy variables are:

- BCVA
- 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 3 grade scoring procedure
- EQ-5D questionnaire
- VFQ25 questionnaire

The secondary safety variables are:

- Adverse events
- 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
- 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.

7. STATISTICAL METHODS

All statistical analyses will be performed using the STATA or SAS® System, latest version available.

7.1. Study Analysis Sets

7.1.1. Analysis Data Sets for the Efficacy Analyses

Full Analysis Set (FAS)

All subjects who have been randomized and for whom the primary efficacy variable is assessed will be included in the FAS. The FAS will be used in all efficacy analyses.

Per-Protocol Analysis Set (PPAS)

All subjects in the FAS without any relevant protocol deviations. The criteria for inclusion in the PPAS will be described in the SAP and documented before code breaking. The PPAS will be used in the analysis of the primary efficacy variable only.

7.1.2. Analysis Data Set for the Safety Analysis

Safety Analysis Set

All subjects who have received at least 1 dose of study medication will be included in the Safety Analysis Set. This set will be used in all analyses of safety data.

7.2. Statistical Analytical Plan (SAP) for Efficacy, Safety and Tolerability Evaluation

A SAP with more technical and detailed elaboration of the principal features of the proposed statistical analyses, presentations, and the way in which anticipated analysis problems will be handled, will be written before the code is broken.

7.3. Demographic Variables and Baseline Characteristics

Demographic variables and baseline characteristics will be summarized in tabular form showing absolute and relative frequencies for categorical variables, and mean, standard deviation, median, 25th and 75th percentiles, minimum and maximum for continuous variables.

7.4. Evaluation of Efficacy Parameters

All efficacy evaluations will be based on the FAS. Moreover, the primary efficacy variable, CRR, will be evaluated also for the PPAS.

7.4.1. Primary Efficacy Variable

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% confidence interval for the difference between the treatments

obtained from the superiority analysis gives the necessary information. Position of the lower end of the confidence interval relative to a pre-defined agreed 0.20 non-inferiority margin (Δ) provides the key information for making decisions (conclusions) about 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 confidence intervals 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 (CPMP) 482/99.

Null hypothesis superiority:

- 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 analyzed with a logistic regression model and model-based estimates of the difference between treatments, odds ratio with corresponding confidence intervals and p-values will be provided. Time-to-event (clinical resolution) analysis will also be performed for the comparison of CRR_12s between treatments, including 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.

7.4.1.1. Justification for choice of the non-inferiority margin Δ

7.4.1.1.1. Justification 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[20], 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 non-inferior*".

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% confidence interval (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 - Delta) / M1		**58%**
The Test treatment retains well over 50% of the Standard treatment effect over placebo		

7.4.1.1.2. Justification 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 anti-amoebics 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 favor of PHMB mono-therapy, will have important beneficial consequences for patients and ophthalmic services.

7.4.2. Secondary Efficacy Variable

Which test to be used in the analyses of secondary efficacy variables depends on the type of variable, e.g., logistic regression for binary variables, proportional odds model for an ordinary scaled variable, analysis of variance (ANOVA) or covariance (ANCOVA) for continuous variables as appropriate. If the underlying assumptions are not fulfilled, data transformation or non-parametric test will be performed. For comparison of proportions, Chi square tests, Fisher's exact test, or Mantel-Haenszel procedures, as appropriate, will be considered. For continuous variables, the Wilcoxon rank-sum test or rank ANOVA/ANCOVA will be used. The STATA or SAS statistical software will be used for the analysis.

EQ-5D and VFQ25 will be evaluated according to their respective analysis protocols.

7.5. Evaluation of Safety and Tolerability Parameters

7.5.1. 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.

7.5.2. Other Safety Parameters

Clinical laboratory tests will be presented with summary statistics. In addition, the tests will be analyzed 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 hypotheses:

- No difference between test treatment and reference treatment

7.6. Sample Size Determination

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.[20] This figure is in the range of cure rates described in the literature.[13,22,23] If the true

difference in CRR_12 is 0.20 (Δ) (or more) in favor 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).

7.7. Interim Analysis

No interim analysis is planned.

8. ADVERSE EVENT REPORTING

8.1. Definitions

8.1.1. Adverse Event

An AE is any untoward medical occurrence in a study subject administered a pharmaceutical product; the event does not necessarily have a causal relationship with the treatment or usage.

AEs include the following:

- Abnormal test findings, as specified below
- Clinically significant signs and symptoms

In addition, signs and symptoms resulting from the following should also be handled according to the same principles as AEs:

- Overdose
- Interactions
- Abuse
- Misuse

8.1.2. Abnormal test findings

The investigator must record all clinically significant abnormal clinical laboratory analysis values during the study in the AE section of the CRF.

Clinical significance is defined as any variation in signs, symptoms, or testing that has medical relevance according to the investigator and may result in an alteration in medical care.

Note, that the symptom, not the test result, should be recorded as an AE.

8.1.3. Pre-existing conditions

Disease signs, symptoms, and/or laboratory abnormalities already existing prior to the start of the study (signing the informed consent) are not considered AEs, unless they re-occur after the subject has recovered from the pre-existing condition or in the opinion of the investigator they represent a clinically significant exacerbation in intensity or frequency.

8.1.4. Procedures

Diagnostic and/or therapeutic non-invasive and invasive procedures, such as surgery, should not be reported as AEs. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an AE. For example, an acute appendicitis that begins during the AE reporting period should be reported as the AE and the resulting appendectomy should be entered in the CRF.

8.1.5. Serious Adverse Events

An AE that meets 1 or more of the following criteria/outcomes is classified as serious:

- Results in death.
- Is life-threatening (i.e., at immediate risk of death).
- Requires in-patient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Is a congenital anomaly/birth defect (i.e., in an offspring to the study subject).

Other medically important AEs that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, that does not result in hospitalization. Any suspected transmission of an infectious agent via the study drug shall also be considered serious.

Serious also includes any other event that the investigator or Sponsor judges to be serious. Some events (such as, for example, corneal vascularization, corneal ulceration, corneal perforation, corneal scarring, non-infectious scleritis) are known to be a consequence of *Acanthamoeba* keratitis and therefore are expected to occur during the study. The investigator is recommended to consult the Investigator's Brochure[10].

8.1.6. Hospitalization

Hospitalization includes transfers within a hospital and also includes admissions of less than 24 hours. The following situations are not considered hospitalizations (although other SAE criteria may still apply):

- Outpatient procedures / ambulatory care.
- Emergency department visits.

Hospitalization in the absence of an AE occurring during the study should not be considered an SAE. This includes:

- Hospitalization due to a pre-existing condition not associated with a worsening of the pre-existing condition.
- Protocol specified admission.
- Elective admission, e.g., due to cosmetic surgery.
- Pre-planned admission for a condition specified at baseline for the subject.

8.2. Adverse Event Reporting Period

The period for recording AEs, including SAEs, on the CRF begins immediately after the subject has signed the informed consent (Visit 1) and ends at the last study visit. SAEs that are related to the study drug and continue beyond the normal collection period (i.e., are ongoing) will be followed until the SAE is resolved or considered medically stable by the investigator. Furthermore, any SAE should be reported irrespective of the time of occurrence if a causal relationship between the event and the study drug is suspected. Data collected after the end-of-study visit will be recorded in the subject's medical record, and in the sponsor's Safety Database Eliciting and Recording Adverse Event Information.

The investigator is to record all directly observed AEs, and all AEs spontaneously reported by the subject, in the CRF using concise medical terminology. In addition, each subject will be questioned about AEs at each clinic visit following initiation of treatment. The question asked will be "Have you had any health problems since your last clinic visit?"

When possible and appropriate, a diagnosis rather than individual signs and symptoms shall be recorded. The investigator is responsible for obtaining sufficient information to determine seriousness, causality and outcome of each AE.

8.2.1. Severity Assessment

The intensity of AEs will be graded using the following scale:

- **Mild (Grade I)**: Asymptomatic or mild symptoms not interfering with the normal activity of the subject: clinical or diagnostic observations only; intervention not indicated.
- **Moderate (Grade II)**: Minimal, local or non-invasive intervention indicated; limited age-appropriate instrumental activities of daily living.

- **Severe (Grade III)**: Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living; incapacitating with inability to work or perform normal daily activity.

- **Life-threatening (Grade IV)**: consequences: urgent intervention indicated.

- **Death (Grade V)** related to AE.

If an AE has multiple aspects, the aspect with the highest severity will be graded.

Note the distinction between the gravity (seriousness) and the intensity (severity) of an AE. Severe is a measure of intensity; thus, a severe reaction is not necessarily a serious reaction. For example, a headache may be severe in intensity, but would not be classified as serious unless it met one of the criteria for serious events listed above.

8.2.2. Causality Assessment

For each AE, the investigator must make a causality assessment to determine if there is a reasonable possibility that the study drug caused the AE.

The relationship of any AE to the study drug will be assessed and graded on a five point scale.

Unrelated: Sufficient information exists to indicate that causality is unrelated to drug i.e. the event is due to extraneous causes (an underlying medical condition, concurrent drugs, environmental factors etc.)

Unlikely: The AE is likely to have been produced by the subject's clinical state, environmental or toxic factors or other therapeutic interventions but an effect of drug cannot be ruled out.

Possible: The AE follows a reasonable temporal sequence from drug administration; is unlikely to have been produced by the subject's clinical state, environmental or toxic factors or other therapeutic interventions.

Probable: The AE follows a reasonable temporal sequence from drug administration, or is associated with established drug concentration in body tissues; improves on stopping or reducing drug dosage (de-challenge); and could not reasonably be explained by the study subject's clinical state environmental or toxic factors, or other therapeutic interventions. (Note: - There are exceptions when an AE does not disappear upon discontinuation of the drug. For example: bone marrow depression, fixed drug eruptions, tardive dyskinesias, etc.).

Related: As for "probable" - but on the basis of the observation of objective clinical signs any other cause may be excluded.

8.3. Serious Adverse Event Reporting

Both serious and non-serious AEs are to be reported on the AE page of the CRF as specified in the CRF instructions.

If an SAE occurs, the investigator or clinical site personnel should notify the Monitor and the sponsor's Qualified Person Responsible for Pharmacovigilance (QPPV) regardless of relationship to the investigational drug, using the designated Serious Adverse Event Form within 24 hours of clinical site personnel becoming aware of the event.

Where the same data are collected in the CRF and on the SAE form, these must be completed in a consistent manner. For example, the same AE term should be used on both forms.

All new information obtained (follow-up information), relevant to an SAE report, should be forwarded to the Monitor and the sponsor's QPPV within the same timeframe as the initial information (within 24 hours from clinical site personnel becoming aware of the event).

The investigator shall provide the Monitor and the sponsor's QPPV with sufficient information to enable a complete medical assessment of the reported event. Best efforts shall be made by the investigator to provide the Monitor and the sponsor's QPPV with additional information related to any SAE as requested.

All SAE reports should be submitted by e-mail or fax to the following contacts:

Daria Rasà
Qualified Person Responsible for Pharmacovigilance, SIFI SpA (ITALY)
Tel +39 095 79 22 204
Mob +39 346 8399737
Fax +39 095 78 93 451
E-mail: **daria.rasa@sifigroup.com**
AND: pharmacovigilance@sifigroup.com
or

Michele Puglia
Deputy QPPV, SIFI SpA (ITALY)
Tel: +39 095 79 22 356
Mob: +39 335 60 77 919
E-mail: **michele.puglia@sifigroup.com**
AND: pharmacovigilance@sifigroup.com

Where required by local regulations, the Institutional Review Board (IRB)/(Independent Ethics Committee (IEC) and/or regulatory authorities will be informed of SAEs in accordance with local regulation timeframes and reporting requirements.

8.4. Exposure during Pregnancy

All events of exposure to the study during pregnancy (female subject) shall be reported to the Monitor and the sponsor's QPPV within 24 hours of awareness by any study personnel, whether the exposure is associated with an AE or not. This includes all situations where a female is or has been found to be pregnant after being exposed to the study drug; directly, indirectly or via her partner (paternal exposure).

In all reported situations of exposure during pregnancy, the sponsor's QPPV will provide the investigator with a Pregnancy Report Form which shall be completed and returned by the investigator. The investigator is responsible for monitoring the outcome of the pregnancy and to inform the Monitor and the sponsor's QPPV of relevant information and any information requested related to the outcome of the pregnancy.

Any AEs and SAEs observed during and in relation to pregnancy or delivery should be recorded in the CRF and as applicable be reported to the Monitor and the sponsor's QPPV as described previously in this section.

8.5. Follow-up of Adverse Events

All AEs should be followed-up until they are resolved or the investigator assesses them as persistent. In particular, all AEs assessed by the investigator as related to the study drug will continue to be followed-up until 28th day after the last application of study drug, even after the subject's participation in the study is completed, or in case of withdrawal of the subject from the clinical trial or of anticipated conclusion of the study.

8.6. Recording

All AEs reported by the subjects or apparent from their physical appearance during the clinical study will be reported in the CRF.

This will include the following information:

- Description of the AE
- Date and time (SAEs only) of onset – End date and time (SAEs only)
- Date and time of last study drug dose for drug related AEs
- Severity of symptoms
- Action taken with study drug
- Action taken (medication)
- AE outcome
- Assessment of relation to study medication
- Optional comments by investigator

In addition, clinically significant abnormal clinical laboratory analysis values should also be recorded as AEs. Reporting as an AE should not be triggered by:

- Merely repeating an abnormal test or

- Any abnormal test result that is determined to be an error.

9. QUALITY CONTROL AND QUALITY ASSURANCE

Monitoring visits to the clinical research center will be made periodically, according to the standard operating procedure of the contract research organization, during the study to ensure that Good Clinical Practice (GCP) and all aspects of the protocol are followed. Source documents will be reviewed for verification of agreement with data on CRFs. The investigator/institution guarantees direct access to source documents by the sponsor and appropriate regulatory authorities.

The clinical research center may also be subject to review by an IEC, to quality assurance audits performed by the sponsor, and/or to inspection by appropriate regulatory authorities.

It is important that the investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

10. ETHICAL ASPECTS

10.1. Independent Ethics Committee/Institutional Review Board

Prospective approval of the study protocol, protocol amendments, informed consent forms, and other relevant documents (e.g., advertisements), if applicable, will be obtained from the IEC/IRB. All correspondence with the IEC/IRB should be retained in the Investigator File. Copies of IEC/IRB approvals should be forwarded to the sponsor.

The only circumstance in which an amendment may be initiated prior to IEC/IRB approval is where the change is necessary to eliminate apparent immediate hazards to the subjects. In that event, the investigator must notify the IEC/IRB and the local site personnel in writing within 5 working days after the implementation.

10.2. Ethical Conduct of the Study

The study will be performed in accordance with International Conference on Harmonization GCP guidelines, the Declaration of Helsinki (adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, June 1964, and subsequent amendments[24]), and applicable local regulatory requirements and laws.

10.3. Subject Information and Consent

All subjects will be given full and adequate verbal and written information regarding the objective and procedures of the study and the possible risks involved. This information must be provided to the subject prior to undertaking any study-related procedure. The subjects must be informed about their right to withdraw from the study at any time. Written subject information, approved by the IEC/IRB, must be given to each subject before any study-related procedure is undertaken. The written subject information must not be changed without prior approval by the sponsor and the IEC/IRB. Furthermore, it is the responsibility of the investigator to obtain signed informed consent.

10.4. Privacy of Personal Data

All personal details will be treated as confidential by the investigator and staff of the research center and handling of personal data will be in compliance with the applicable local laws and regulations.

11. STUDY ADMINISTRATIVE STRUCTURE

11.1. Documentation

11.1.1. Archiving

All documents concerning the study will be kept on file in the Central Archives of the sponsor for at least 25 years after study completion and completion of the Clinical Study Report. The sponsor will receive the completed CRFs and the final database in SAS.

11.1.2. Case report form

A CRF is required and should be completed for each included subject. In this study an electronic CRF will be used, which is fully ICH-GCP/EU Clinical Trial Directive compliant..

It is the responsibility of the investigator to ensure completion and to review and approve all CRFs. CRFs must be signed by the investigator, using an electronic signature. These signatures serve to attest that the information contained on these CRFs is correct. At all times, the investigator has final responsibility for the accuracy and authenticity of all data entered on the CRFs.

11.1.3. Source data

Subject source documents are the physician's subject records maintained at the clinical research center. In most cases, the source documents will be the hospital's or the physician's chart. In those cases, the information collected on the CRFs must match those charts. All reports and printouts should be stored in the subject's medical record.

12. CONFIDENTIALITY AND PUBLICATION POLICY

By signing this protocol the investigator reaffirms to the sponsor that he/she will maintain in confidence all information furnished to him/her, or resulting from this study. He/she will only divulge such information as may be necessary to the IEC and the members of the staff and the subjects who are involved in this study.

The results of this study may be published or presented at scientific meetings. If this is envisaged, both parties agree to inform each other and to submit all manuscripts or abstracts to the other party prior to publication or presentation.

Prior notice of any planned publication shall be given to the other parties concerned at least 45 days before the publication. Any objection to the planned publication shall be made in writing to the sponsor and to any party concerned within 30 days after receipt of the notice. If no objection is made within the time limit stated above, the publication is permitted. An objection is justified if

(a) The objecting Party's legitimate academic or commercial interests are compromised by the publication; or

(b) The protection of the objecting Party's Foreground or Background is adversely affected.

The objection has to include a precise request for necessary modifications.

If an objection has been raised the involved parties shall discuss how to overcome the justified grounds for the objection on a timely basis (for example by amendment to the planned publication and/or by protecting information before publication) and the objecting Party shall not unreasonably continue the opposition if appropriate actions are performed following the discussion.

This allows the parties concerned to protect proprietary information and to provide comments based on information that may not yet be available to the other party.

Authorship of any formal publication of the study will be determined by mutual agreement between the investigator and the sponsor.

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APPENDIX 1 TYPICAL CLINICAL FINDINGS OF AN *ACANTHAMOEBA* CORNEAL INFECTION

1. epithelial lesions: epithelial punctate keratopathy, epithelial infiltrates, epithelial defects, dendritiform epithelial ulcers
2. extracorneal lesions: limbal inflammation (limbitis), anterior scleral inflammation, diffuse or nodular
3. stromal lesions: perineural infiltrates, anterior stromal infiltrates, disciform corneal swelling, stromal ulceration, ring abscess
4. anterior chamber lesions: keratic precipitates, hypopyon
5. iris lesions: fixed dilated pupil
6. lens: mature cataract

APPENDIX 2 EUROQOL FIVE-DIMENSION SCALE (EQ-5D) QUESTIONNAIRE

Health Questionnaire

English version for the UK

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems 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 doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

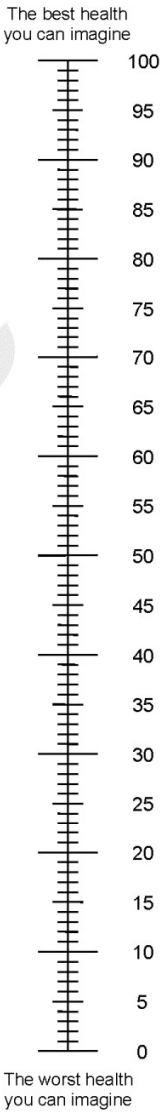
ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

2

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



3

APPENDIX 3 VISUAL FUNCTIONING QUESTIONNAIRE 25 (VFQ25)

PB/SA

National Eye Institute
Visual Functioning Questionnaire - 25
(VFQ-25)

version 2000

(SELF-ADMINISTERED FORMAT)

January 2000

- 1 - ***version 2000***

The following is a survey with statements about problems which involve your vision or feelings that you have about your vision condition. After each question please choose the response that best describes your situation.

Please answer all the questions as if you were wearing your glasses or contact lenses (if any).

Please take as much time as you need to answer each question. All your answers are confidential. In order for this survey to improve our knowledge about vision problems and how they affect your quality of life, your answers must be as accurate as possible. Remember, if you wear glasses or contact lenses, please answer all of the following questions as though you were wearing them.

INSTRUCTIONS:

1. In general we would like to have people try to complete these forms on their own. If you find that you need assistance, please feel free to ask the project staff and they will assist you.

2. Please answer every question (unless you are asked to skip questions because they don't apply to you).

3. Answer the questions by circling the appropriate number.

4. If you are unsure of how to answer a question, please give the best answer you can and make a comment in the left margin.

5. Please complete the questionnaire before leaving the center and give it to a member of the project staff. Do not take it home.

6. If you have any questions, please feel free to ask a member of the project staff, and they will be glad to help you.

STATEMENT OF CONFIDENTIALITY:

All information that would permit identification of any person who completed this questionnaire will be regarded as strictly confidential. Such information will be used only for the purposes of this study and will not be disclosed or released for any other purposes without prior consent, except as required by law.

- 1 - ***version 2000***

Visual Functioning Questionnaire - 25

PART 1 - GENERAL HEALTH AND VISION

1. In general, would you say your overall health is:

(Circle One)

Excellent	**1**
Very Good	**2**
Good	**3**
Fair	**4**
Poor	**5**

2. At the present time, would you say your eyesight using both eyes (with glasses or contact lenses, if you wear them) is excellent, good, fair, poor, or very poor or are you completely blind?

(Circle One)

Excellent	**1**
Good	**2**
Fair	**3**
Poor	**4**
Very Poor	**5**
Completely Blind	**6**

- 2 - ***version 2000***

3. How much of the time do you worry about your eyesight?

(Circle One)

None of the time	**1**
A little of the time	**2**
Some of the time	**3**
Most of the time	**4**
All of the time?	**5**

4. How much pain or discomfort have you had in and around your eyes (for example, burning, itching, or aching)? Would you say it is:

(Circle One)

None	**1**
Mild	**2**
Moderate	**3**
Severe, or	**4**
Very severe?	**5**

PART 2 - DIFFICULTY WITH ACTIVITIES

The next questions are about how much difficulty, if any, you have doing certain activities wearing your glasses or contact lenses if you use them for that activity.

5. How much difficulty do you have reading ordinary print in newspapers? Would you say you have:

(Circle One)

No difficulty at all	**1**
A little difficulty	**2**
Moderate difficulty	**3**
Extreme difficulty	**4**
Stopped doing this because of your eyesight	**5**
Stopped doing this for other reasons or not interested in doing this	**6**

- 3 - ***version 2000***

6. How much difficulty do you have doing work or hobbies that require you to <u>see well up close</u>, such as cooking, sewing, fixing things around the house, or using hand tools? Would you say:

(Circle One)

No difficulty at all	**1**
A little difficulty	**2**
Moderate difficulty	**3**
Extreme difficulty	**4**
Stopped doing this because of your eyesight	**5**
Stopped doing this for other reasons or not interested in doing this	**6**

7. Because of your eyesight, how much difficulty do you have <u>finding something on a crowded shelf</u>?

(Circle One)

No difficulty at all	**1**
A little difficulty	**2**
Moderate difficulty	**3**
Extreme difficulty	**4**
Stopped doing this because of your eyesight	**5**
Stopped doing this for other reasons or not interested in doing this	**6**

8. How much difficulty do you have <u>reading street signs or the names of stores</u>?

(Circle One)

No difficulty at all	**1**
A little difficulty	**2**
Moderate difficulty	**3**
Extreme difficulty	**4**
Stopped doing this because of your eyesight	**5**
Stopped doing this for other reasons or not interested in doing this	**6**

9. Because of your eyesight, how much difficulty do you have <u>going down steps, stairs, or curbs in dim light or at night</u>?

- 4 - ***version 2000***

(Circle One)

No difficulty at all	**1**
A little difficulty	**2**
Moderate difficulty	**3**
Extreme difficulty	**4**
Stopped doing this because of your eyesight	**5**
Stopped doing this for other reasons or not interested in doing this	**6**

10. Because of your eyesight, how much difficulty do you have noticing objects off to the side while you are walking along?

(Circle One)

No difficulty at all	**1**
A little difficulty	**2**
Moderate difficulty	**3**
Extreme difficulty	**4**
Stopped doing this because of your eyesight	**5**
Stopped doing this for other reasons or not interested in doing this	**6**

11. Because of your eyesight, how much difficulty do you have seeing how people react to things you say?

(Circle One)

No difficulty at all	**1**
A little difficulty	**2**
Moderate difficulty	**3**
Extreme difficulty	**4**
Stopped doing this because of your eyesight	**5**
Stopped doing this for other reasons or not interested in doing this	**6**

- 5 - ***version 2000***

12. Because of your eyesight, how much difficulty do you have <u>picking out and matching your own clothes</u>?

(Circle One)

No difficulty at all	**1**
A little difficulty	**2**
Moderate difficulty	**3**
Extreme difficulty	**4**
Stopped doing this because of your eyesight	**5**
Stopped doing this for other reasons or not interested in doing this	**6**

13. Because of your eyesight, how much difficulty do you have <u>visiting with people in their homes, at parties, or in restaurants</u> ?

(Circle One)

No difficulty at all	**1**
A little difficulty	**2**
Moderate difficulty	**3**
Extreme difficulty	**4**
Stopped doing this because of your eyesight	**5**
Stopped doing this for other reasons or not interested in doing this	**6**

14. Because of your eyesight, how much difficulty do you have <u>going out to see movies, plays, or sports events</u>?

(Circle One)

No difficulty at all	**1**
A little difficulty	**2**
Moderate difficulty	**3**
Extreme difficulty	**4**
Stopped doing this because of your eyesight	**5**
Stopped doing this for other reasons or not interested in doing this	**6**

15. Are you <u>currently driving</u>, at least once in a while?

- 6 - ***version 2000***

(Circle One)

Yes..... 1 *Skip To Q 15c*

No 2

15a. IF NO: Have you <u>never</u> driven a car or have you <u>given up driving</u>?

(Circle One)

Never drove..... 1 *Skip To Part 3, Q 17*

Gave up..... 2

15b. IF YOU GAVE UP DRIVING: Was that <u>mainly because of your eyesight</u>, <u>mainly for some other reason</u>, or because of <u>both your eyesight and other reasons</u>?

(Circle One)

Mainly eyesight..... 1 *Skip To Part 3, Q 17*

Mainly other reasons..... 2 *Skip To Part 3, Q 17*

Both eyesight and other reasons .... 3 *Skip To Part 3, Q 17*

15c. IF CURRENTLY DRIVING: How much difficulty do you have <u>driving during the daytime in familiar places</u>? Would you say you have:

(Circle One)

No difficulty at all..... 1
A little difficulty..... 2
Moderate difficulty..... 3
Extreme difficulty..... 4

- 7 - ***version 2000***

16. How much difficulty do you have <u>driving at night</u>? Would you say you have:

(Circle One)

No difficulty at all	**1**
A little difficulty	**2**
Moderate difficulty	**3**
Extreme difficulty	**4**
Have you stopped doing this because of your eyesight	**5**
Have you stopped doing this for other reasons or are you not interested in doing this	**6**

16A. How much difficulty do you have <u>driving in difficult conditions, such as in bad weather, during rush hour, on the freeway, or in city traffic</u>? Would you say you have:

(Circle One)

No difficulty at all	**1**
A little difficulty	**2**
Moderate difficulty	**3**
Extreme difficulty	**4**
Have you stopped doing this because of your eyesight	**5**
Have you stopped doing this for other reasons or are you not interested in doing this	**6**

- 8 - ***version 2000***

PART 3: RESPONSES TO VISION PROBLEMS

The next questions are about how things you do may be affected by your vision. For each one, please circle the number to indicate whether for you the statement is true for you all, most, some, a little, or none of the time.

	(Circle One On Each Line)				
READ CATEGORIES:	**All of the time**	**Most of the time**	**Some of the time**	**A little of the time**	**None of the time**
17. Do you accomplish less than you would like because of your vision?	**1**	**2**	**3**	**4**	**5**
18. Are you limited in how long you can work or do other activities because of your vision?.....	**1**	**2**	**3**	**4**	**5**
19. How much does pain or discomfort in or around your eyes, for example, burning, itching, or aching, keep you from doing what you'd like to be doing? Would you say:	**1**	**2**	**3**	**4**	**5**

- 9 - ***version 2000***

For each of the following statements, please circle the number to indicate whether for you the statement is definitely true, mostly true, mostly false, or definitely false for you or you are not sure.

(Circle One On Each Line)

	Definitely True	Mostly True	Not Sure	Mostly False	Definitely False
20. I stay home most of the time because of my eyesight.	**1**	**2**	**3**	**4**	**5**
21. I feel frustrated a lot of the time because of my eyesight.	**1**	**2**	**3**	**4**	**5**
22. I have much less control over what I do, because of my eyesight.	**1**	**2**	**3**	**4**	**5**
23. Because of my eyesight, I have to rely too much on what other people tell me...	**1**	**2**	**3**	**4**	**5**
24. I need a lot of help from others because of my eyesight.	**1**	**2**	**3**	**4**	**5**
25. I worry about doing things that will embarrass myself or others, because of my eyesight.	**1**	**2**	**3**	**4**	**5**

LAST PAGE