25 July 2024
EMA/286425/2024, Rev.1[1]
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Akantior

International non-proprietary name: polihexanide

Procedure No. EMEA/H/C/005858/0000

[1] Revision 25 July 2024

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List of abbreviations

Quality

ASMF	Active Substance Master File = Drug Master File
CFU	Colony Forming Units
FT-IR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromatography
GC-MS	Gas chromatography mass spectrometry
GPC	Gel permeation chromatography
HDPE	High Density Polyethylene
HPLC	High performance liquid chromatography
IC	Ion chromatography
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICP-MS	Inductively coupled plasma mass spectrometry
LC	Liquid chromatography
LDPE	Low Density Polyethylene
MS	Mass Spectrometry
NLT	Not less than
NMT	Not more than
Ph. Eur.	European Pharmacopoeia
RH	Relative Humidity
RP	Reversed Phase
RRT	Relative retention time
SmPC	Summary of Product Characteristics
UHPLC	ultra-high performance liquid chromatography
UV	Ultraviolet

Non-clinical

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ASMF	Active Substance Master File
AST	Aspartate aminotransferase
ATP	Adenosine tripohosphate
AUC	Area under the concentration-time curve
CAS No.	Chemical Abstracts Service Registry number
CHMP	Committee for Medicinal Products for Human Use
Cmax	Maximum observed concentration
CTD	Common Technical Document
BKC	Benzalkonium chloride
DNA	Desoxyribonucleic acid
ECHA	European Chemical Agency
EFD	Embryo-foetal development
EMA	European Medicines Agency
GLP	Good Laboratory Practice
HCE	Human corneal epithelium
LC-MS/MS	Liquid chromatography coupled with tandem mass spectrometry
NOAEL	No observed adverse effect level
NOAEC	No observed adverse effect concentration
MAA	Marketing authorization application
MAC	Minimum amoebicidal concentration
MCC	Minimum cysticidal concentration
MW	Molecular weight
NOAEL	No observed adverse effect level
Ph. Eur	European Pharmacopeia

PCR	Polymerase chain reaction
PHMB	Polihexanide
RAC	Committee for Risk Assessment
SmPC	Summary of Product Characteristics
Tmax	Time to reach maximum observed concentration
USP	United States Pharmacopeia

Clinical

AE	Adverse event
AK	*Acanthamoeba* keratitis
ANOVA	Analysis of variance Analysis
ANCOVA	Analysis of covariance
ATC	Anatomic therapeutic class
BCVA	Best corrected visual acuity
CDC	Centres for Disease Control and Prevention
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval
CR	Clinical resolution
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 randomisation, defined as the percentage of subjects cured at 30 days after discontinuing all study therapies within 12 months of randomisation
DLE	Dose Limiting Event
EC	European Commission
EMA	European Medicines Agency
EQ-5D	EuroQol five-dimension scale
EU	European Union
FAS	Full Analysis Set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good manufacturing practice
HSV	Herpes simplex virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
INN	International Nonproprietary Name
IOP	Intraocular pressure
IRB	Institutional Review Board
IUD	Intrauterine device
IUS	Intrauterine hormone releasing system+
ITT	Intention-To-Treat
IUPAC	International Union of Pure and Applied Chemistry
IVCM	*In vivo* Confocal Microscopy
LAM	Lactational amenorrhoea method
LAR	Legally authorized representative
LogMAR	Log of the Minimum Angle of Resolution
MAA	Marketing Authorization Application
MCC	Minimum cysticidal concentration

MTAC	Minimum trophozoite amoebicidal concentration
NOAEL	No Observed Adverse Effect Level
NSAID	Non-steroidal anti-inflammatory drugs
ODAK	Orphan Drug for *Acanthamoeba* keratitis
PCR	Polymerase chain reaction
PD	Pharmacodynamic(s)
PDCO	Paediatric Committee
PHMB	Polyhexamethylene biguanide, Polihexanide
PK	Pharmacokinetic(s)
PPAS	Per-Protocol Analysis Set
PP	Per protocol
PSF	Product Specification File
PSR	PSR Group BV (now Ergomed) (the CRO)
PT	Preferred Term
Q	Quartile
qid	Quarter in die (four times a day)
QPPV	Qualified person responsible for pharmacovigilance
RR	Relative risk
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SAS	Safety Analysis Set
SD	Standard deviation
SOC	System organ class
TEAEs	Treatment Emergent Adverse Events
UK	United Kingdom
US	United States
USP	United States Pharmacopeia
VAS	Visual analog scale
VFQ 25	Visual functioning questionnaire 25

* This is a general list of abbreviations. Not all abbreviations may be used

1. Background information on the procedure

1.1. Submission of the dossier

The applicant SIFI SPA submitted on 2 May 2022 an application for marketing authorisation to the European Medicines Agency (EMA) for Akantior, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 29 January 2021.

Akantior was designated as an orphan medicinal product (EU/3/07/498) on 14 November 2007 in the following condition: treatment of *Acanthamoeba* keratitis.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Akantior as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: https://www.ema.europa.eu/en/medicines/human/EPAR/Akantior

The applicant applied for the following indication: treatment of *Acanthamoeba* keratitis in adults and children from 12 years of age.

1.2. Legal basis and dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent full-mixed application.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain tests or studies).

1.3. Information on paediatric requirements

Not applicable on the basis that *Acanthamoeba* keratitis is extremely rare in this population and clinical studies were considered not to be feasible (P/0134/2017).

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.4.2. New active substance status

The applicant requested the active substance polihexanide contained in the above medicinal product to be considered as a new active substance, as they claimed that it is not a constituent of a medicinal product previously authorised within the European Union.

Based on the review of available data on the active substance, the CHMP considered that polihexanide was not to be qualified as a new active substance in itself as it is a constituent of a medicinal product previously authorised within the European Union. Polihexanide is contained in the products Serasept 1 (MA no. 57861.00.00) and Serasept 2 (MA nfo. 57862.00.00), authorised in Germany (with different indications from Akantior).

The applicant thus withdrew the NAS request.

1.5. Protocol assistance

The applicant received the following Protocol assistance on the development relevant for the indication subject to the present application:

Date	**Reference**	**SAWP co-ordinators**
26 June 2008	EMEA/H/SA/1064/I/2008/PA/III	*Armin Koch, Andrea Laslop*
24 July 2014	EMEA/H/SA/1064/2/2014/PA/III	*Mair Powell, Brigitte Blöchl-Daum*
23 June 2016	EMEA/H/SA/1064/2/FU/1/2016/PA/III	*Karl-Heinz Huemer, Minne Casteels, Markku Pasanen*

On 26 June 2008, the applicant received advice EMEA/H/SA/1064/1/2008/PA/III pertaining to the following *non-clinical, and clinical* aspects:

- Non-clinical: minimum cycsticidal concentrations (MCCs), *in vivo* and *in vitro* models
- Clinical assessment of PK in man, dose ranging approach, the phase III study design, use of a historical control in phase III concerning dose finding and safety assessment, the phase III study design including population, comparator, masking, primary endpoint, follow up, duration, safety database and statistical aspects.

On 24 July 2014, the applicant received advice, EMEA/H/SA/1064/2/2014/PA/III pertaining to *quality, non-clinical, and clinical*.

- Quality: "B" criteria of the Ph. Eur. of preservatives, and GMP aspects
- Non-clinical: the update of the non-clinical development plan.
- Clinical: paediatric patients, update protocol and statistical aspects for the pivotal clinical study, Conditional Approval.

On 23 June 2016, the applicant received advice EMEA/H/SA/1064/2/FU/1/2016/PA/III pertaining to *quality, non-clinical, and clinical*

- Multidisciplinary: ocular PK study in rabbits.
- Clinical. Clinical PK data, the proposed dosage regimen for the Phase III study, the phase III study protocol.

1.6. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Daniela Philadelphy Co-Rapporteur: Jayne Crowe

The application was received by the EMA on	2 May 2022
The procedure started on	19 May 2022
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	8 August 2022
The CHMP Co-Rapporteur's critique Assessment Report was circulated to all CHMP and PRAC members on	22 August 2022
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	22 August 2022
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	15 September 2022
The applicant submitted the responses to the CHMP consolidated List of Questions on	08 September 2023
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	16 October 2023
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	26 October 2023
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	9 November 2023
The applicant submitted the responses to the CHMP List of Outstanding Issues on	25 March 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	19 April 2024
The CHMP agreed on a list of second outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	25 Apr 2024
The applicant submitted the responses to the 2nd CHMP List of Outstanding Issues on	2 May 2024
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Akantior on	30 May 2024
Revised CHMP Opinion A revised opinion was adopted by the CHMP in order to critically review the inherent coherence within the report and the findings that led the CHMP to conclude that the benefit-risk balance of Akantior is positive	25 July 2024

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Acanthamoeba keratitis is a rare, painful, and sight-threatening infection caused by *Acanthamoeba* species, a family of free-living, ubiquitous protozoa commonly found in water, dust, and soil. This infection results from invasion of ocular tissue through a corneal lesion.

2.1.2. Epidemiology and risk factors

Acanthamoeba keratitis can affect people of all ages. However, most people who contract the infection are young or middle-aged and otherwise healthy, with the main predisposing factor being contact lens use (Randag et al 2019). The incidence of *Acanthamoeba* keratitis is described to be between 1 and 4 per million (Nielsen et al 2019, Randag et al 2019). The publication of the Dutch registry identified an estimated incidence rate of 1 in 21,000 soft contact lens users (Randag et al 2019), equating to 1 to 3.5 per million in the general population. The incidence of *Acanthamoeba* keratitis has increased rapidly over recent years (Nielsen et al 2019, Randag et al 2019, List et al 2021). The incidence of *Acanthamoeba* keratitis over a year is seasonal with most patients becoming infected during warmer months. This may be driven by people traveling to warmer climates or participating in aquatic leisure activities. Higher levels of *Acanthamoeba* are found in surface water during warmer months (List et al 2021).

2.1.3. Biologic features, aetiology and pathogenesis

Several different species and genotypes of *Acanthamoeba* have been recognized, all exist in two forms: an active form (trophozoite) and a dormant form (cyst). The trophozoite is the active form of *Acanthamoeba*; it can reproduce by binary fission in optimal growth conditions and cause human infections. In unfavourable conditions, *Acanthamoeba* trophozoites can transform into cysts; this is the dormant state of *Acanthamoeba* characterised by a very low metabolic rate but resistance to environmental challenges. Under favourable conditions, cysts transform gradually into the trophozoite and the trophozoites emerge from cyst through channels called ostioles, leaving an empty double-wall shell. The cystic form of *Acanthamoeba* is responsible for persistent disease.

Damage starts at the moment *Acanthamoeba* enters the eye, proliferates and starts feeding from the cornea. This combined with a natural inflammatory responses causes corneal vascularisation, scarring and corneal perforation. Extra-corneal complications such as scleritis, retinal necrosis, cataract, glaucoma and iris atrophy can also occur. As a consequence, *Acanthamoeba* keratitis can require single or multiple corneal transplants to alleviate symptoms or restore vision once infection is eliminated.

2.1.4. Clinical presentation and diagnosis

Diagnosis and treatment of *Acanthamoeba* infection are difficult. Early diagnosis and prompt delivery of appropriate medical therapy are essential to secure a good prognosis. If effective therapy is delayed by three weeks or more, prognoses deteriorate (Varacalli et al 2021). A delayed diagnosis may cause deeper corneal involvement with severe sequelae requiring more intensive treatment, including surgery. *Acanthamoeba* keratitis is specifically more likely in case of keratitis occurring in contact lens

wearers or corneal trauma complicated by exposure to soil or contaminated water. Confirmation of infection can be achieved via cytological staining after corneal scarping, *in vivo* confocal microscopy (IVCM), culture of corneal scraping, polymerase chain reaction (PCR) and histology of corneal biopsies. If left untreated, the condition may frequently result in blindness, or the need for corneal transplant surgery. The goal of *Acanthamoeba* keratitis therapy is the removal of *Acanthamoeba* cysts and trophozoites from corneal tissue and the resolution of inflammation (Maycock et al 2016). The treatment course to reach these goals is often long and challenging, and while the trophozoite form is susceptible to multiple therapies, the cystic form is highly drug resistant and may persist for months.

2.1.5. Management

There are currently no drugs licensed for use in *Acanthamoeba* keratitis (AK) and no standard treatment is established across the EU. Thus, patients and ophthalmologists use unlicensed or off-label treatment options. Diamidines and biguanides are considered effective cysticidal anti-amoebic agents. The current approach for the treatment for *Acanthamoeba* keratitis includes biguanides (e.g. polihexanide or chlorhexidine) with or without the addition of diamidines (e.g. propamidine or hexamidine). These products (including polihexanide, propamidine, chlorhexidine and hexamidine), synthesised from time to time in laboratories/ pharmacies in the EU, although such manufacturing has not yet been carried out in compliance with GMP, are also used for the ocular treatment of AK, albeit not being approved for this indication.

According to literature, in most cases additional concomitant therapy is applied (i.e. especially antibiotics, antifungals, corticosteroids and pain killers). In cases of medical failure, a surgical intervention (as amniotic membrane transplantation, deep anterior lamellar keratoplasty or penetrating keratoplasty) may be required in approximately one third of patients (Varacalli et al 2021; Robaei et al 2015; Randag et al 2019; List et al 2021).

Early diagnosis and immediate medical care is required to alleviate acute symptoms of infection and to reduce infection load (i.e. density of *Acanthamoeba*) as soon as possible, in order to prevent major damage to the patients cornea and ultimately to maintain patients vision / prevent vision loss.

2.2. About the product

2.3. Type of Application and aspects on development

The CHMP did not agree to the applicant's request for an accelerated assessment as the product was not considered to be of major public health interest. This was based on the fact that treatment options (including polihexanide) currently used in the clinical practice, also in medical grade, are sufficiently stable for long-term storage, despite not being licensed for AK. Additionally, the increased concentration of polihexanide in the proposed product was not perceived as a major therapeutic innovation compared to the currently applied therapy. Furthermore, available data from the completed clinical phase III study showed that polihexanide 0.8 mg/mL is not superior to the chosen comparator. Additionally, the results do not allow to unambiguously conclude on a beneficial therapeutic value of a high concentrated polihexanide formulation compared to a combination of polihexanide and propamidine. Therefore, the applicant's claim of "substantial improvement of efficacy" was not supported by their pivotal study versus the comparator chosen among the products currently used as best supportive care for ethical reasons.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as eye drops, solution in single-dose container containing 0.8 mg/mL of polihexanide (PHMB). Polihexanide is a polymeric active substance in the hydrochloride salt form. Each single-dose container is filled with 0.3 mL of solution. The average drops size volume is 31.8 µl corresponding to approximately 25.4 µg of polihexanide per eye drop.

Other ingredients are: sodium dihydrogen phosphate monohydrate, disodium phosphate dodecahydrate, sodium chloride and purified water.

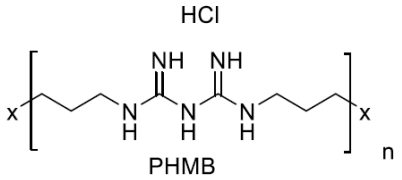
The product is available in low density polyethylene (LDPE) single-dose containers as described in section 6.5 of the SmPC.

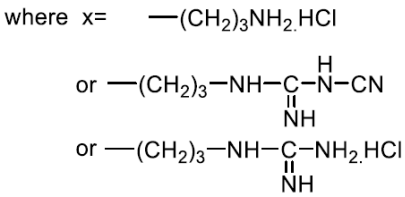
2.4.2. Active substance

2.4.2.1. General information

The chemical name of polihexanide is homopolymer of *N*-(3-aminopropyl)-imidodicarbonimidic diamide. The active substance is also known under the name polyhexamethylene biguanide hydrochloride (polihexanide). The active substance is a polymer for which the molecular formula corresponds to $(C_8H_{17}N_5)_n \cdot x$ HCl with n equal to the number of monomer units (average molecular weight). The weight-average molecular weight (Mw) corresponds to 2300-6000 Da. The active substance has the following structure:

Figure 1. active substance structure





A broad set of relevant analytical methods have been used to verify and characterise the structure of the active substance. The chemical structure of polihexanide was elucidated by a combination of infrared spectroscopy, ultraviolet spectroscopy, nuclear magnetic resonance spectroscopy, elemental analysis, gel permeation chromatography, chloride ion content analysis, differential scanning

calorimetry (DSC), thermal gravimetric analysis and polarised light microscopy (PLM). Gel permeation chromatography was used to determine the molecular weight of the active substance as well as the polymer dispersity index. The active substance is an amorphous solid which is evidenced by the observation of multiple endothermic peaks in the DSC curve and further confirmed by the PLM spectrum. Structural elucidation was complemented with mass spectrometry (HPLC-TOF) to characterise oligomers in the active substance. Three different end groups of the polymeric active substance were identified: amine, cyanoguanide and guanide. The groups at chain end were quantified by nuclear magnetic resonance spectroscopy.

The active substance polihexanide is an amorphous white solid, which is hygroscopic and freely soluble in water across the pH range. Polihexanide has a non - chiral molecular structure.

2.4.2.2. Manufacture, characterisation and process controls

Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF and it was considered satisfactory.

The active substance is manufactured by one manufacturing site (Supplier Y).

Polihexanide is synthesised in a few steps using well defined starting materials with acceptable specifications.

The applicant's part of the ASMF includes a general flow chart and a very brief narrative description of the synthesis and purification of polihexanide active substance. The absence of a chemical reaction scheme was accepted, as starting materials and chemical steps are described in sufficient detail for this relatively simple route of synthesis.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of active substances.

Potential and actual impurities are well discussed with regards to their origin and characterised.

Finished product batches used for clinical studies (Phase I, III and pivotal studies) were manufactured using active substance produced by a manufacturer which is different from the manufacturer proposed for commercial manufacturing. This is further discussed in the finished product section below.

The active substance is packaged in double low-density polyethylene (LDPE) bags (an inner LDPE bag sealed with a cable tie encased within a heat-sealed LDPE bag), encased within a heat-sealed aluminium foil outer bag, and then packed within a HDPE keg. The packaging of the active substance ensures protection from exposure to moisture. The primary packaging material complies with Commission Regulation (EU) 10/2011, as amended.

2.4.2.3. Specification

The active substance specification includes tests for: appearance (visual), identification (FT-IR, GPC), biguanide assay (titration), assay on anhydrous basis (RP-HPLC) related substances (LC-UV and LC-MS), residual HMD (IC), total impurities (UPLC-UV and UPLC-MS), residual solvents (headspace GC, RP-HPLC), chloride ion content (titration), microbial limits (Ph. Eur.), water content (KF), sulphated ash (Ph. Eur.) and elemental impurities (ICP-MS).

The active substance specification is acceptable and the justification for the proposed specification is acceptable and in line with ICH Q6A. The specification used by the finished product manufacturer is identical to the release specification of the active substance shown above as used by the active substance manufacturer with the exception of testing for elemental impurities, which is only performed by the active substance manufacturer. The analytical methods used are also identical.

Based on administration instructions for the finished product as defined in the SmPC (maximum 16 drops per eye, i.e. 32 drops per day) and a drop size of 31.8 µL, the maximum daily dose (MDD) of polihexanide active substance is calculated as 0.8 mg. Corresponding thresholds according to ICH Q3A are: reporting threshold 0.05%, identification threshold 0.10% and qualification threshold 0.15%. An acceptable toxicological justification has been provided for those impurities where the respective limit is above the Ph. Eur. qualification threshold (0.15%).

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines.

During the procedure, a major objection was initially raised as the control of the active substance was not considered sufficient. In response, an additional test was added to the active substance specification to quantify the active substance content (assay on anhydrous basis by RP-HPLC). In addition, a new test method for related substances (gradient UPLC-MS and -UV) was developed which is able to separate and quantify specified related substances. Furthermore, all specified related substances are now tested separately with individual limits, as requested. Limits are accepted based on batch data and process capability. Based on results using non-clinical/clinical batches, there is no safety concern regarding these impurities. The revised active substance specification includes a reference (footnote) clarifying that qualified impurity limits are only applicable for the tested route of administration (ophthalmic use) and maximum daily dose (MDD = 0.896 mg). Regarding identification of two impurities, the presented efforts for identification are regarded as sufficient. A most-probable structure has been presented for both compounds, which are by-products of the polymerisation step as isolation of these compounds proved difficult. The limit for a specified impurity was tightened, as requested. The limit for total impurities has been tightened as requested. The issues raised in the major objection related to the control of the active substance have been resolved.

The in-house GPC method used to confirm the identity of the active substance determines molecular weight and the polydispersity index using PEG standards. Based on the data provided, the proposed acceptance criteria for molecular weight and polydispersity index are acceptable.

Several specification limits have been tightened, as requested during the procedure and are now acceptable.

Elemental impurities (class 1, class 2A, Sb, Cu and Li) are routinely tested in the active substance at a level of 10% of the respective parenteral permitted daily exposure.

Batch analysis data from six commercial-scale batches of the active substance are provided. All batches were manufactured by the proposed commercial manufacturer (Supplier Y) in 2020 and 2021. These batches include three validation batches which were manufactured according to the proposed commercial process. All batch results were within the specification. Systematic differences in molecular weight determined by the external testing site used by the finished product manufacturer and results obtained by the active substance manufacturer for the same active substance batches have been sufficiently explained.

A tabular overview of critical process parameters (CPPs) applied for the three scale-up and three process validation batches is provided. Although different propositions were made for the two manufacturing campaigns (information available in the restricted part of the ASMF), CPPs actually

applied can be regarded as similar. In conclusion, the six batches can be regarded as representative for the commercial process.

Based on the process data presented, the critical process parameters for the polymerisation conditions were set appropriately.

Satisfactory information regarding the reference standards used for assay and impurities testing has been presented. PEG standards used for the determination of molecular weight and polydispersity index have been characterised.

The applicant's Part of the ASMF was amended with a comprehensive justification of the active substance specification including representative batch data. Comparative data on assay and related substances of six commercial active substance batches (Supplier Y) and NC/C batches (manufactured by Supplier X) obtained with the newly developed analytical methods were submitted. The level of impurities was comparable. The level of by-products is lower in the commercial active substance. Notably, the new related substances method was able to detect several unspecified impurities which were not found in the commercial active substance.

2.4.2.4. Stability

Stability data from six commercial-scale batches of active substance (three full-scale stability and three validation) batches from the proposed manufacturer stored in a container closure system representative of that intended for the market for up to 24 months under long term conditions (25 ºC / 60% RH) and for up to six months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. For the accelerated stability studies, residual HMD was tested for the validation batches only. All tested parameters were compliant with the initially submitted specification. Newly developed methods for assay and related substances (see above) were not applied (testing period 12/2020 to 06/2021). For the long-term stability studies, data obtained with validation batches include two time-points (18 months and 24 months) with duplicate results, i.e. results according to the initial specification and results including new assay and impurities methods. All tested parameters were compliant with the initially submitted specification. Results obtained at 18 months and 24 months were compliant with the updated specification. No data from accelerated stability studies on assay (content of active substance) and related substances (individual results for all specified impurities, unspecified and total impurities) are available. This is regarded acceptable in this particular case as new analytical methods were developed during the procedure and because no extrapolation of data is proposed. Long-term data according to the revised specification (including results for assay active substance and individual impurity levels) are available up to time point 24 months. The analytical methods used were the same as for release and were stability indicating. The stability-indicating nature of the UHPLC-MS method was demonstrated by forced degradation studies. The obtained mass balance was acceptable. The forced degradation studies show degradation of the active substance under acidic and basic aqueous conditions through hydrolysis. It was further confirmed that the GPC method to determine the molecular weight and PDI is stability indicating.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 24 months when stored at 15-25°C and protected from exposure to moisture.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

Akantior eye drops, solution in single-dose container is a sterile solution for ocular administration. Each container is for single use only and must be used immediately after opening. Akantior presents as a clear and colourless solution which is practically free of visible particles. The solution has a pH of 5.6 – 6.0 and an osmolality of 270 – 330 mOsmol/kg. Each mL of solution contains 0.8 mg of the active substance polihexanide corresponding to 0.08% w/w. Each vial contains 300 µl of solution. The average drops size volume is 31.8 µl corresponding to approximately 25.44 µg of polihexanide per eye drop. The single-dose containers are moulded in 5-unit sealed strips (supplied in an outer pouch or sachet).

Satisfactory information has been presented on the development of the medicinal product.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards, with the exception of disodium phosphate dodecahydrate, which complies with USP. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC and in paragraph 2.1.1 of this report. Purified water is used. This is in line with EMA/CHMP/CVMP/QWP/496873/2018 and thus acceptable. The choice of excipients, their concentration and their function are briefly discussed. The formulation does not contain preservatives. No overages are used.

The key physicochemical characteristics of the active substance which can influence the performance of the finished product are discussed. These are molecular weight, polydispersity, content of biguanide and other functional groups (e.g. amino, cyanoguanidino, guanidino) and protonation of the polycation (pK).

The compatibility of the active substance with the excipients used is confirmed by stability studies (see below).

The formulation of the proposed solution containing 0.8 mg/mL polihexanide is based on an earlier formulation containing 0.2 mg/mL polihexanide, which was qualitatively and quantitatively the same. Studies to develop the buffer system and osmolality have been performed with the 0.2 mg/mL formulation. The information on formulation development is satisfactory.

Finished product batches used for clinical studies (Phase I, III and pivotal studies) were conducted using a 0.8 mg/mL polihexanide solution produced by a manufacturer which is different from the manufacturer proposed for commercial manufacturing. The active substance polihexanide is a polymeric molecule with varying molecular weight which can contain different functional groups at the end of the chain. The structure and purity of polihexanide strongly depend on the polymerisation conditions. Based on the information available, it is not possible to clearly determine or state the actual polihexanide content in the batches used for non-clinical and clinical studies. Consequently, similarity in terms of content of the active substance cannot be concluded at the level of quality comparison. The molecular structure of active substance from the supplier of clinical material (Supplier X) is different from the molecular structure of active substance manufactured by the proposed commercial manufacturer (Supplier Y). Differences are noted with regard to the ratio of in-chain guanide and biguanide units. Potential differences in terminal groups (cyanoguanide, guanide and amine) cannot be evaluated based on the data provided as the end groups have not been quantified for active substance used in clinical trials and no further retrospective analysis is possible. From a quality point of view, similarity of finished product used in non-clinical and clinical studies (Supplier X) and commercial finished product (Supplier Y) cannot be concluded. The impact of the differences observed as regards

quality aspects (molecular structure, polihexanide assay) on safety and efficacy of the finished product cannot be evaluated based on physico-chemical parameters. Due to the absence of suitable retain samples of finished product containing active substance supplied by Supplier X, further investigations are not feasible. Hence, from a quality perspective this issue cannot be resolved. It is noted as a matter of fact that, a) apparent differences between finished product used in clinical trials and commercial finished product exist regarding the structure of the active substance and b) similarity cannot be investigated for assay polihexanide due to absence of suitable finished product samples containing active substance supplied by Supplier X. In conclusion, the potential impact of the existing differences on safety and efficacy cannot be evaluated by quality data. As a consequence, clinical data obtained with finished product containing active substance from Supplier X cannot be bridged to the commercial finished product containing active substance from the proposed commercial active substance manufacturer Supplier Y based on quality data. The major objection raised in this regard during the procedure could not be resolved at quality level. The applicant responded to the major objection by providing non-clinical data therefore reference is made to the non-clinical and clinical assessment below on the impact of the differences in quality attributes on clinical safety and efficacy.

As the finished product is intended for administration upon the eyeball, the physicochemical parameters considered most relevant (pH and osmolality) have been studied. Efficacy of the finished product is strongly dependent on positive charge of the polycation in solution. The viscosity of the finished product has also been discussed. Due to the low concentration of the active substance, it is assumed that formation of micelles can be excluded.

A summary on the manufacturing process development has been provided. The manufacturing process was developed for the 0.2 mg/mL formulation. As the type of manufacturing process is regarded as well-established for ophthalmic solutions in single-dose containers, the information provided is sufficient. However, the proposed sterilisation and aseptic manufacturing process is generally accepted for ophthalmic products due to the patient benefit of the proposed container closure system (single use). Therefore, the initially raised major objection is considered resolved.

Results from a drop size study have been presented. Drop sizes from two operators and from three batches were studied as regards drop weight. The effect of the orientation of the primary packaging (inverted (90°) or inclined (45°)) was also studied.

The usability of the finished product in the proposed container is supported by clinical trials. Reference is made to the clinical section (see below).

Results from an extractables and a leachables study have been presented. An extractables study was performed using three extracting solvents (water solution at pH 4.3, water solution at pH 7.3 and a mixture of water solution/ethanol 80/20 v/v). Samples were stored at 60°C±2°C for 72 h±1 h. Leachables have been investigated in the finished at the end of its shelf-life. This was done to evaluate the presence in the finished active products of substances coming from primary packaging material kept under normal storage conditions. Neither non-volatile, nor semi-volatile nor volatile organic extractables have been found above the safety threshold (AET of 0.28 µg/test item). The analytical methods used are described in sufficient detail. The results are acceptable.

As regards microbiological attributes, the finished product is sterile and meets the requirements for sterility set out in Ph. Eur. 2.6.1.

The primary packaging is low density polyethylene (LDPE) single-dose containers. The LDPE does not contain additives. The material complies with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product. The container closure system is considered a standard material for ophthalmic solutions

filled in single-dose units. Each single-use LDPE 5-unit strip is contained in a polyester/ aluminium/ polyethylene pouch.

2.4.3.2. Manufacture of the product and process controls

The finished product is manufactured by one manufacturing site (SIFI S.p.A, Italy).

The main steps of the manufacturing process consist of: preparation of the finished solution, sterilisation and packaging/labelling. The process is considered to be a non-standard manufacturing process.

The manufacturing process has been adequately described.

Sufficient information on process parameters (times, temperature, pressure, quantities of used materials and pH ranges) is provided. The mean fill weight is controlled by in-process control.

A sound discussion regarding sterilisation of the container closure system material is presented and the information is satisfactory.

Manufacturing hold times are defined. The bulk solution is tested for microbiological purity.

Critical process steps and critical process parameters have been adequately defined.

The functional qualities of the plastic containers are controlled by in-process controls.

Major steps of the manufacturing process have been validated by a number of studies. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form. Process validation was performed with three commercial batches (50 kg each). Validation data is provided for several in-process controls showing that pH, osmolality, appearance, tests on bulk solution, tests during filling were consistent within the three tested batches. Compliance of critical process parameters is demonstrated by means of batch data.

Viability of the test for microorganisms in the bulk solution is demonstrated. Results from bacterial retention test are seen as adequate.

2.4.3.3. Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: appearance (Ph. Eur.), colour (Ph. Eur.), water loss (gravimetric), mean fill weight (gravimetric), particulate contamination (Ph. Eur.), pH (Ph. Eur.), osmolality (Ph. Eur.), identify polihexanide (HPLC, UV), assay polihexanide (HPLC), impurities (HPLC) and sterility (Ph. Eur.).

The finished product release specification is acceptable and covers all relevant parameters for the dosage form.

During the procedure, a major objection was initially raised on the control of the finished product and the analytical methods used. In response, new analytical methods have been developed and satisfactory information on the validation of these methods has been provided. To test for assay of polihexanide, a new gradient RP-HPLC method has been developed. The new analytical procedure has been validated according to ICH Q2(R1) for the following parameters: specificity (chromatograms are provided), robustness, stability, linearity and range (80-120% of test concentration), accuracy (80-120% of test concentration), repeatability and intermediate precision. This was accepted by the CHMP.

To test for related substances, two new HPLC-UV methods have been developed which replace the initially used test method for detection and quantification of related substances. One method is used for determination of some impurities (both controlled under the parameter any other unidentified impurity). The analytical procedure has been validated according to ICH Q2(R1) for the following parameters: specificity (chromatograms are provided), robustness, stability, LOQ, LOD, linearity and range, accuracy, repeatability and intermediate precision. The second method is responsible for determination of some other impurities. The analytical procedure has been validated according to ICH Q2(R1) for the following parameters: specificity (chromatograms are provided), robustness, stability, LOQ, LOD, linearity and range, accuracy, repeatability and intermediate precision. Both methods are used for the detection and quantification of total impurities. With the implementation of the two new analytical methods, the initially raised issues regarding related to impurity detection and quantification are now adequately addressed and the major objection is resolved. Both methods have been shown to be stability-indicating.

The suitability of the microbiological method for sterility testing according to Ph. Eur. was demonstrated.

The potential presence of elemental impurities in the finished product has been assessed following a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Option 2a (component approach with a specified daily intake) has been applied. All used materials (active substance, excipients, equipment, containers, processing aids, primary packaging materials) and the manufacturing process itself have been taken into account. A worst-case scenario has been investigated as all elements mandatory for the parenteral route have been investigated. Based on the risk assessment it can be concluded that it is not necessary to include any elemental impurity controls.

A risk assessment concerning the potential presence of nitrosamine impurities in the finished product has been performed as requested considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). In addition, results from confirmatory testing were also presented. Based on the information provided, it is accepted that there is no risk of nitrosamine impurities in the active substance or the related finished product. Therefore, no specific control measures are deemed necessary. The initially raised major objection is resolved.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are provided for three commercial-scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

The finished product is released on the market based on the above release specifications, through traditional final product release testing.

2.4.3.4. Stability of the product

Stability data from three commercial-scale batches of finished product stored for up to 24 months under long term conditions (25 ºC / 40% RH) and for up to 6 months under accelerated conditions (40 ºC / NMT 25% RH) according to the ICH guidelines were provided. The batches of medicinal product are representative to those proposed for marketing and were packed in the primary packaging

proposed for marketing. The analytical procedures used are stability indicating. No significant changes have been observed. The applied conditions are in line with the stability guideline for finished products in semipermeable containers.

Stability data was also provided for finished product batches containing active substance provided by Supplier X, but these are seen as supportive data only due to the comparability issues discussed above.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. Samples in the primary packaging and also in the primary packaging inside the aluminium pouch have been investigated. No difference or degradation has been observed. The finished product is therefore regarded as photostable.

In-use stability studies

In-use stability studies have been conducted whereby the aluminium pouch has been opened and the strips (composed of five single-use containers) have been left inside mimicking the possible exposure to oxygen/light of the solution in the semipermeable containers. Samples were tested at day 0, day 14 and day 28. The new methods developed to control assay and impurities in the finished product have been used. All results remained within specification.

Based on available stability data, the proposed shelf-life of 2 years without special storage conditions as stated in the SmPC (sections 6.3 and 6.4) is acceptable. Once the outer sachet has been opened, the single-dose containers must be used within 28 days (after this period, any unused single-dose containers must be discarded). The contents of the single dose container must be used immediately after opening.

2.4.3.5. Adventitious agents

No excipients derived from animal or human origin have been used.

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner.

During the procedure, five major objections were initially raised on quality aspects, relating to 1) the control of the active substance, 2) the comparability of active substance and finished product used in clinical trials and in non-clinical studies with the active substance and finished products proposed for marketing, 3) the use of sterilization method, 4) the control of the finished product and the analytical methods used and 5) the risk of presence of nitrosamines. All major objections were resolved as discussed. As regards major objection 2 on comparability, this issue cannot be resolved at quality level. Reference is made to the non-clinical and clinical assessment and the benefit-risk assessment of the product, where the totality of the data has been considered.

The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.4.6. Recommendation(s) for future quality development

Not applicable.

2.5. Non-clinical aspects

2.5.1. Introduction

The applicant developed Akantior 0.8 mg/mL eye drops (solution in single-dose container), which is a polihexanide (polyhexamethylene biguanide hydrochloride) based product for topical ocular use. This product is indicated for the treatment of *Acanthamoeba* keratitis. Polihexanide is a polymeric biguanide comprising a polycationic linear polymer with a hydrophobic backbone and multiple cationic groupings separated by hexamethylene chains.

2.5.2. Pharmacology

The active substance polihexanide is a well-known and ubiquitously used disinfectant, antiseptic and biocidal agent. The mode of action of polihexanide against *Acanthamoeba* has been well investigated, and indeed polihexanide is used 'off-label' in treating patients suffering from *Acanthamoeba* keratitis.

2.5.2.1. Primary pharmacodynamic studies

In terms of primary pharmacodynamics, only three pharmacology studies were originally submitted in Module 4.2.1 (SIFI Study No. PC1301_PHMB-DNA BINDING ASSAY_2106 and the literature studies of Sudano Roccaro and Asero 2020 and Asero et al. 2015). After the first round of assessment, the Applicant additionally submitted the Favennec 2023 study, being the full study report of the conference paper Asero et al. 2015. In another round, the Applicant submitted additional *in vitro* pharmacology studies (Study No. UoB-0124-Pt1&2 and the supplementary Study SIFI Study No. 24001).

At first, the current state of the literature on the primary pharmacodynamics is summarised and discussed, then summaries of the submitted pharmacology studies are provided.

In the pharmacology written summary in Module 2.6.2, the Applicant compiled results of *in vitro* and *in vivo* literature studies. Specifically, the authors of these studies aimed at evaluating the amoebicidal efficacy of polihexanide (or combinations of polihexanide and another pharmacologically active agent).

The amoebicidal and cysticidal mode of action of polihexanide in *Acanthamoeba* keratitis has been established in a multitude of non-clinical (*in vitro* and *in vivo*) and clinical literature studies (Varacalli et al., 2021; DOI: 10.3390/jcm10050942). Furthermore, polihexanide is being used in an off-label manner in the treatment of *Acanthamoeba* keratitis, either in monotherapy or in combinations with e.g. a topical diamidine such as propamidine isethionate 0.1% (Varacalli et al., 2021; DOI: 10.3390/jcm10050942). The applicant summarises that two properties of polihexanide act amoebicidal and cysticidal: At first disruption of *Acanthamoeba* cell membranes by electrostatic binding of the positively charged polihexanide to the negatively charged cell membranes of *Acanthamoeba* (causing

membrane damage and ultimately cell lysis), and second polihexanide-mediated DNA complexation and chromosome condensation (Rusciano et al 2013).

Out of 10 *in vitro* studies referenced in this submission, the Applicant concluded that eight studies demonstrated good efficacy of polihexanide. For example, the study by Elder et al 1994 demonstrated good trophozoite minimum amoebicidal concentrations and especially minimal cysticidal concentrations of polihexanide compared to 12 other drug candidates. Importantly, in the *in vitro* study of Tirado-Angel et al 1996, the efficacy of polihexanide and chlorhexidine was synergistically increased when both drugs were combined. In the study of Burger et al 1994, efficient killing of two *Acanthamoeba* species by polihexanide was reported. However, no comparator treatments were included in this study. Khunkitti et al 1996 investigated the efficacy of different biocides on *Acanthamoeba* castellanii and established that polihexanide was – together with chlorohexidine - the most efficient amoebicidal agent tested in this study. In the study of Khunkitti et al 1998, the authors demonstrated that polihexanide more effectively caused structural membrane damage to *Acanthamoeba* castellanii trophozoites than chlorohexidine. Lee et al 2007 showed a better cysticidal efficacy of polihexanide compared to chlorohexidine in *Acanthamoeba* castellanii and A. lugdunesis, albeit this effect was not statistically significant. In the study by Moon et al 2015, polihexanide proved effective against *Acanthamoeba* castellanii; however, efficacy was higher when polihexanide was administered together with the cellulose synthesis inhibitors, 2,6-dichlorobenzonitrile and isoxaben. Finally, Shi et al 2020 analysed the efficacy of nine anti-amoebic agents on *Acanthamoeba* castellanii trophozoites and cysts *in vitro*. In this study, polihexanide was similarly effective as propamidine isethionate, natamycin and povidone iodine, and clearly more effective than chlorhexidine, hexamidine diisethionate, dibromopropamidine-diisethionate and miltefosine.

Furthermore, the Applicant compiled minimum amoebicidal concentrations (MAC) and minimum cysticidal concentrations (MCC) of polihexanide from different literature studies, as summarised in the table below:

Table 1. Comparative effectiveness of polihexanide against *Acanthamoeba* isolates reported in the literature

Test item	Number of tested strains	MAC (µg/mL) mean value	MAC (µg/mL) range	MCC (µg/mL) mean value	MCC (µg/mL) range	Reference
Polihexanide	13 (MAC); 23 (MCC)	1.3	0.49-3.9	2.2	0.49-3.9	Elder *et al* 1994
	19	-	-	55	25-100	Narasimhan *et al* 2002
	20	1.6	0.8-6.3	6.3	3.2-12.5	Seal 2003

Abbreviations: MMC = minimum cysticidal concentration; MAC = minimum amoebicidal concentration.

These references support the amoebicidal and cysticidal efficacy of polihexanide at the proposed posology of Akantior.

However, *in vitro* and *in vivo* literature studies only demonstrated weak or even lacking amoebicidal efficacy of polihexanide. Furthermore, literature studies suggest that combination therapies of polihexanide and an additional pharmacologically active agent could be more effective than a polihexanide monotherapy.

The applicant also submitted own studies in Module 4. For example, in the publication of Sudano Roccaro 2020, the Applicant studied whether:

1.) an ATP bioluminescence assay for the viability determination of *Acanthamoeba* cysts can be compared with the traditional hemocytometry analysis;

2.) a range of cysticidal concentrations of polihexanide are effective in the treatment of *Acanthamoeba*;

3.) the cysticidal activity of polihexanide is affected by its molecular weight;

4.) polihexanide produced by Supplier X and Supplier Y have comparable cysticidal efficacy at comparable molecular weight (MW).

With regards to the first and second endpoint of this study, a good linear correlation (R2 = 0.9900) was found between the cyst count number and the RLU emitted by amoebae, whereby effectivity of polihexanide was demonstrated at a range of different concentrations (specifically between 0.008% to 0.0002% of polihexanide in solution). In regards to the third endpoint of this study, the Applicant demonstrated that a specific range of molecular weights of polihexanide showed identical amoebicidal activity at 0.0008%. Of note, the batches of these different molecular weight polihexanide specimens were obtained from different manufacturers. Finally, in regards to the amoebicidal activity of polihexanide batches produced by Supplier X and by Supplier Y that shared a similar molecular weight, the Applicant demonstrated identical cysticidal activity of both polihexanide products despite their origin from different manufacturers. However, at this point reference is made to the quality assessment of Akantior, in which a major objection was originally raised due to the lack of adequate comparability studies between the polihexanide batches produced by Supplier X and Supplier Y.

Additionally, the Applicant conducted an *in vivo* study in a rat model of *Acanthamoeba* keratitis (Favennec 2023). In this *Acanthamoeba* polyphagia keratitis rat model, the anti-trophozoic efficacy of different polihexanide concentrations (0.02%, 0.04%, 0.06% and 0.08% corresponding to 0.2, 0.4, 0.6 and 0.8 mg/mL, respectively) was tested. Of note, also a combination treatment group of polihexanide 0.02% and propamidine 0.1% and a vehicle control group was included. In this study the Applicant concluded that 0.04% and 0.06% polihexanide significantly prevented corneal lesions worsening between day 14 and 28 compared to the control group. The same efficacy was observed with 0.08% polihexanide (however, this group failed to achieve statistical significance). The polihexanide + propamidine combination treatment proved less efficient in terms of clinical efficacy than the polihexanide mono-treatments. However, in terms of extent of decreases in cultures, histology and PCR evaluations, all test-article treatments delivered approximately similar results of efficacy. Importantly, no consistent dose-response relationship in the different efficacy endpoints was observed in this study in the polihexanide 0.02%, 0.04%, 0.06% and 0.08% treatments.

In response, the Applicant conducted new comparative *in vitro* efficacy studies with polihexanide batches manufactured by Supplier Y and new batches manufactured by Supplier X (manufactured in Dec. 2022, thus no batches studied in clinical trial 043 were included in this *in vitro* bridge):

The applicant presented a new study from the University of Birmingham (Study No. UoB-0124-Pt1&2) in which Supplier Y DS and Supplier X DS were used on *Acanthamoeba* polyphaga and *Acanthamoeba* castellanii trophozoites and cysts, and in which minimum trophozoite amoebicidal concentrations (MTAC), minimum cysticidal concentrations (MCC), and timekill curves were examined. In order to establish the MTAC and MCC, 10 different dilutions (2-fold each) were tested starting from 1000 µg/ml (0.1%) to 0.97 µg/ml (0.000097%) on both trophozoites and cysts ($1x10^4$/mL each). For trophozoites and cysts of both *Acanthamoeba* species, identical MTAC (7.81 µg/mL) and MCC values (1.95 µg/mL) were obtained with the polihexanide batches of both manufacturers, demonstrating similarity (CAVE: the polihexanide concentration in Akantior is 0.8 mg/ml, or 0.08%). In the timekill kinetics part of the experiment, trophozoites and cysts ($5x10^4$/mL each) were exposed to both Supplier Y and Supplier X DS at the MTAC (i.e 7.8 µg/mL) value and also at the target polihexanide concentration in Akantior (i.e

0.8 mg/mL) for up to 24 h (i.e 0, 1, 2, 4, 6 and 24 h). No statistically significant differences were found between Supplier Y and Supplier X DS at all evaluated timepoints at the two tested concentrations for trophozoites. For cysts, no statistically significant differences were observed between Supplier Y and Supplier X DS apart from 2h in *Acanthamoeba* castellanii and 6h in *Acanthamoeba* polyphaga (Supplier Y DS was superior in killing cysts than Supplier X DS at both time points). While Supplier Y DS tended to perform better than Supplier X DS, no differences were observed between the two polihexanide DS when tested at the commercial Akantior concentration at 0.08% (0.8 mg/mL). Considering the results of this experiment, the Applicant concluded that this study allows to *in vitro* bridge the efficacy between the used Supplier X and Supplier Y DS in killing *Acanthamoeba* trophozoites and cysts.

Of note, no *in vivo* data were generated and no batches of Supplier X that were studied in clinical trial 043 were included in this *in vitro* bridge, leaving some uncertainty regarding a possible extrapolation of *in vitro* results to the clinical setup, as well as the applicability of these results to Supplier X batches that were used during clinical studies.

The applicant did not justify the clinical dose on the basis of the non-clinical pharmacology studies. The proposed clinical dose of 0.8 mg/ml (800 µg/ml) is multiple times higher than the mean minimum cysticidal concentration (MCC) of polihexanide against *Acanthamoeba* species. Thus, efficacy and safety of Akantior should be based on clinical assessment.

2.5.2.2. Secondary pharmacodynamic studies

No secondary pharmacodynamics and safety pharmacology studies were submitted. As Akantior is topically applied on affected eyes in patients suffering from *Acanthamoeba* keratitis (rendering systemic effects unlikely), and as polihexanide is a well-established antiseptic and disinfectant that is also used on an off-label basis, this is acceptable.

2.5.2.3. Pharmacodynamic drug interactions

No pharmacodynamics drug interaction studies were submitted. However, as several literature studies were found that demonstrated that combinations of polihexanide and a second pharmacologically active agent can enhance efficacy in the treatment of *Acanthamoeba* keratitis, and also as concomitant ocular therapies are described in the treatment of this disease, the lack of pharmacodynamics drug interaction studies is reflected in the SmPC. Principally, additive and/or synergistic and antagonistic pharmacodynamic drug interactions are conceivable. It is reported that a concomitant medication is being used in the treatment of Acanthamoeba keratitis. Systemic absorption of polihexanide after use of Akantior is negligible or not detectable. Possible implications for Akantior are discussed in more detail in the clinical assessment sections below.

2.5.3. Pharmacokinetics

No absorption, distribution, metabolism and excretion studies after topical ocular administration were submitted.

However, two studies were submitted in Module 4.2.2.7, specifically Study 15114 and Study 97240. The goal of both studies was to set up an analytical method that is capable of determining the ocular and subsequently systemic PK of polihexanide when topically administered to rabbits. Specifically, two appointed CROs (RTC and Intertek) were not able to set up a reliable mass spectrometry method (specifically UPLC or LC-MS/MS) that could measure polihexanide in relevant biological media. In fact, both methods failed as no suitable hydrolysis protocol was found that could guarantee reliable cleavage

into the monomers and dimers of polihexanide. Considering that the polihexanide polymer is characterised by a wide range of different molecular weights (if not degraded to well defined units like monomers or dimers), polihexanide could not be reliably quantitated by mass spectrometry in biological media.

However, the authors in Vontobel et al 2015 reported that in an *ex vivo* study (using rabbit corneas clamped in artificial perfusion chambers), penetration of polihexanide through corneas with intact epithelium into the artificial anterior chamber was not detected during the 8 hours of the study. Even with mechanical epithelial debridement of the corneas, no penetration was detected in this study, which might be due to electrostatic attraction between the positively charged polihexanide and negatively charged corneal proteoglycans.

The applicant subsequently submitted an additional *ex vivo* PK study: A new transcorneal permeation study using an ex vivo drug release test in porcine eyes in the Franz cell apparatus (SIFI Study No. PC1301_EX VIVO TRANSCORNEAL PERM. STUDY_2301). In this ex vivo study, the Applicant found that no permeation of polihexanide and fluorescein (internal control) was detected across the porcine cornea. Importantly, it should however be mentioned that this experiment might only imperfectly represent ocular conditions during *Acanthamoeba* keratitis under which corneal barrier function might be compromised and absorption kinetics therefore enhanced.

In addition to these studies and literature references, in the toxicology written summary (submitted in Module 2.6.6), the Applicant reported the results of three oral rat toxicokinetics studies, which also contained biodistribution endpoints, and in which radiolabelled polihexanide was administered (SCCS 2015). For example, in a rat PK study in which Alpk:APf SD (Wistar derived) rats were used, up to 10% of polihexanide were excreted via urine after oral administration, demonstrating that a fraction of the orally administered polihexanide was resorbed from the gastrointestinal tract, distributed to the kidneys and ultimately excreted via urine. Furthermore, in one of these rat studies, the highest amounts of retained radioactivity were found in the liver (0.18% of dose in males and 0.19% of dose in females) and kidneys (0.03% of dose in males and 0.04% of dose in females). This indicates that only little of the administered polihexanide was retained in organs (but also that polihexanide concentrations in organs were indeed measurable). Furthermore, in one of these studies, the residual carcasses contained up to 1.3% of the administered dose, demonstrating that some of the administered polihexanide has long excretion times or might even accumulate to a certain extent. In another rat study, the tissues of the gastrointestinal tract retained 0.02 % - 0.1% of the administered radioactivity. Finally, in a study in which Alderley Park rats were used, some of the orally administered polihexanide-related radioactivity was also exhaled (specifically 0.2%).

With regards to metabolism of polihexanide, the Applicant reported from one of the oral rat PK literature studies that after administration of radiolabelled polihexanide to male Alderley Park rats, the constituents of polihexanide were not metabolized (SCCS 2015).

Finally, no pharmacokinetic drug interaction studies were submitted. Thus, it remains uncertain whether potential local interactions (e.g. on physical-chemical level) might occur. A statement to inform that no interaction studies have been performed was inserted in section 4.5 of the SmPC. The uncertainty appears sufficiently addressed by informing the intended users. Also, the instruction for a 5-minute interval between administrations of ophthalmic products outlined in the PI is acknowledged. It is agreed that systemic interactions are not expected, as systemic exposure is negligible.

2.5.4. Toxicology

Three repeated dose toxicity studies (specifically Study 8898, Study A1272 and Study A2018) and two *in vitro* local tolerance studies (specifically Study ODAK_cytotoxicity_1301 and Study ODAK-

cytotoxicity_1302) were originally submitted. In all other parts of the toxicology dossier (e.g. genotoxicity, carcinogenicity, reproductive and developmental toxicity), reference was made to literature studies. Later during the assessment, the Applicant submitted two additional *in vitro* safety studies (Eurofins Study No. STULV24AA0004-1 GLP; Eurofins Study No. STULV24AA0003-1 GLP; SIFI Study No. PC1301_IRRITATION_ 2401).

2.5.4.1. Single dose toxicity

With regards to single dose toxicity, the Applicant summarised *in vivo* literature studies in which LD_{50} values of polihexanide were reported, i.e. after oral and dermal administration. Oral LD_{50} values in rats ranged from 25.6 mg/kg to 1049 mg/kg. After dermal administration, LD_{50} values in rats and NZW rabbits were higher than 400 mg/kg. Considering that only 0.8 mg/mL are administered per eye and per hour (16 times per day in total), that many of the administered polihexanide will be rinsed out the eye and eventually be washed off, and that only little of the topically applied polihexanide will reach the systemic circulation, the expectable exposure to polihexanide after Akantior administration will be orders of magnitude below these LD_{50} values. Therefore, it is not thought that acute toxicity after Akantior administration will pose a relevant risk to patients.

2.5.4.2. Repeat dose toxicity

Three repeated dose toxicity studies (Study A1272, Study 8869 and A2018) were submitted in which Akantior was topically administered to the eyes of NZW rabbits. In these studies, the Applicant assessed the local ocular tolerance and the potential systemic toxicity of Akantior when topically administered to eyes. All three studies were conducted in compliance with the OECD GLP regulations. Specifically, a 2-week (Study A1272), a 4-week (Study 8869) and a pivotal 26-week study (Study A2018) were carried out. A safety factor of 10 was applied in study A1272, in the two other studies no safety factor was applied. In the pivotal repeated dose toxicity study, the clinical dose (0.8 mg/mL) was administered at the same posology as applied for Akantior patients.

The objectives of the first repeated dose toxicity study (Study 8869) were to test the ocular tolerance and systemic toxicology of polihexanide when repeatedly administered to eyes of NZW rabbits. Specifically, polihexanide was administered for 28 days to 4 male and female rabbits at 0.005% and 0.02%. Of note, only sub-clinical exposures were applied; i.e. single 0.8 mg/mL (0.08%) drops are applied per hour to *Acanthamoeba* keratitis patients, but in this study only 0.05 and 2 mg/mL were applied per drop and eye. Furthermore, up to 16 drops are applied in the clinical treatment of *Acanthamoeba* keratitis per eye and day, whereas maximally 8 drops per day were applied in this study. Consequently, the exposures in this study do not reach the expected exposures in *Acanthamoeba* keratitis patients. Therefore, this study only bears little toxicological relevance and can thus only be regarded as supplementary information in this application procedure. No toxicologically relevant findings were noted in this study, demonstrating that the applied polihexanide concentrations were well tolerated.

In the second repeated dose toxicity study (Study A1272), the Applicant assessed the ocular tolerance of Akantior when topically administered to approximately 11 weeks old NZW rabbits at 0.08%, 0.25% and 0.8% (being equivalent to 0.8, 2.5 and 8 mg/mL). Per group, four rabbits (male and female) were treated exclusively in the right eye. Administration proceeded for 2 weeks, whereby 50 µL of the test article were administered for 13 times per day at approximately 1 hour intervals during the first week and for 7 times per day during the second week. After the active administration phase, a subset of animals (n=2 per sex of the vehicle and high dose group) was kept for 1 week to study the potential recovery of identified effects.

At 0.08%, slight conjunctival redness and conjunctival discharge were noted in test-article groups. In animals of the 0.25% group, slight chemosis, slight conjunctival redness and slight to moderate discharge was identified. In both groups, reversal of these test-article related effects was observed during the course of the experiment. Despite being test-article related, these effects were characterised to not be of toxicological relevance by the study director, which appears to be supported by the rapid recovery of the observed effects.

However, in the high-dose 0.8% groups (having a 10-fold higher polihexanide concentration per volume unit as is contained in Akantior), moderate to severe test-article related effects were identified that generally increased in severity during the course of the study. Specifically, administration of 0.8% solutions of polihexanide induced slight to moderate conjunctival redness and discharge, slight to moderate chemosis, slight to severe iris inflammation (with congestion of iris vessels observed in the slit lamp examination), slight to complete corneal opacity, slight to severe corneal staining in the fluorescein staining examination, red and swollen conjunctiva, and microscopically apparent slight to mild conjunctival oedema, corneal oedema, acute inflammation, necrosis, iris congestion and acute inflammation. Generally no recovery of the observed ocular effects was apparent in the 0.8% groups throughout the experiment and during the one week recovery period.

The applicant concluded that within the administration scheme used in Study A1272, a 0.08% and 0.25% polihexanide solution did not lead to relevant adverse ocular effects. However, at 0.8%, mild to severe ocular effects were observed that were unequivocally related to test-article administration. However, the dose level in this group was 10-fold higher than the polihexanide concentration contained in Akantior. As no relevant ocular effects were observed in this study at 0.08% in group 1 (i.e. the polihexanide concentration in the clinically used preparation), and as no relevant ocular effects were identified in study A2018 (in which polihexanide was administered at the same posology and concentration as intended for Akantior), it is considered that the findings of the high dose groups in Study A1272 could only be of relevance for patients in situations of high overdoses.

Finally, in the pivotal repeated dose toxicity study (Study A2018), the Applicant assessed the ocular tolerance of polihexanide at 0.8 mg/mL when topically applied to the eyes of NZW rabbits at the clinically intended posology. Administration lasted 26 weeks. In total, 8 male and female rabbits were used in the test article group. The control group was treated at the same posology, but with vehicle only. Rabbits were approximately 13 weeks of age at study initiation. The test-article or the vehicle was administered exclusively to the right eye, the left eye of the animals remained untreated and was used as a control. No notable differences were observed in ocular and systemic endpoints when comparing animals from the vehicle and the test-article groups. At the beginning of the study and on an occasional basis thereafter, slight conjunctival redness was observed in most animals of the test-article groups, and slight discharge was observed at the beginning of the study in some females of the test-article group. These are all expected side effects of the administration of an irritating substance (such as polihexanide) to the eye. However, the severity of these findings was mild. Furthermore, these findings were apparently rapidly recovered, underlining their low risk potential.

Initially, the Applicant calculated safety margins on a µg/animal and µg/kg basis comparing topical administration in rabbits to chronic oral administration in rats. Considering that the Applicant claimed there is little systemic exposure, this choice of calculating margins was not accepted. Assumptions based on volume of eyeball concluded that there is limited absorption across the cornea (Vontobel et al 2015). Surface area would, however, have been more appropriate, considering that Akantior is a topical product.

Following the D120 LoQ, the Applicant compared the pivotal 26-week toxicity study in rabbits to the pivotal clinical trial (Clinical Study Report 043/SI). Both studies administered polihexanide eye drops at 0.08%. This is a more appropriate method, and the calculated safety margin of 1.35 can be accepted.

2.5.4.3. Genotoxicity

The applicant compiled results of various *in vitro* and *in vivo* genotoxicity studies conducted with polihexanide (Johnson et al. 2020). Specifically, the Applicant compiled the results of three Ames tests, of two *in vitro* mouse lymphoma assays, of one *in vitro* micronucleus assay, of one *in vivo* micronucleus assay (C57BL/6JfCD-1/Alpk mice) and of one unscheduled DNA synthesis assay in Wistar-derived rats. The total weight of evidence in these studies demonstrates that polihexanide is non-genotoxic. While isolated positive results were observed in some of the studies, these results were sometimes not reproducible (e.g. in the Ames tests), or were of low magnitude at considerable supratherapeutic concentrations. Furthermore, the topical ocular route of administration of Akantior renders a possible systemic genotoxicity of polihexanide extremely unlikely. Considering all these aspects, it is agreed that a potential risk for genotoxicity of Akantior is presumably negligible.

2.5.4.4. Carcinogenicity

The applicant summarised the results of two long-term rodent studies conducted with polihexanide (Johnson et al. 2020). Specifically, the first study was an oral 124 weeks experiment carried out with female rats (n=60 per group) of unspecified strain. Polihexanide concentrations up to 2000 ppm were administered. The second study was an oral 2 years C57Bl/10J/CD-1/Alpk mouse study (n=55 per group) in which up to 4000 ppm (corresponding to approximately 715 mg/kg/day) were administered daily to exclusively female mice. Of note, the first of these studies is of questionable relevance as infections were observed in the animals (causing increased mortality). In both studies, haemangiomas and haemangiosarcomas were identified at increased albeit low incidence in test-article animal groups. Interestingly, this was consistently observed both in the mouse and in the rat study. The applicant concluded that "*based on the results provided, RAC of ECHA concluded that classification as Carc 2 H351 (suspected of causing cancer) according to the CLP would be appropriate*". However, it is not thought that carcinogenicity is a relevant risk after topical ocular administration of little amounts of polihexanide for a confined time period, as is the case in the proposed treatment of *Acanthamoeba* keratitis. This predominantly stems from the potentially low to negligible systemic polihexanide exposure after Akantior administration to *Acanthamoeba* keratitis patients. Even though no non-clinical and clinical studies have been submitted that evaluated systemic exposure after topical ocular Akantior administration, it is nonetheless considered extremely unlikely that systemic exposure could reach a magnitude that would favour carcinogenesis to a relevant extent. Finally, it is noted that endothelial neoplasms of the vasculature (haemangiomas and haemangiosarcomas) are common background findings in rodent carcinogenicity studies, but are exceedingly scarce in humans (e.g. Cohen et al. 2009, DOI: 10.1093/toxsci/kfp131).

2.5.4.5. Reproductive and developmental toxicity

The applicant compiled the results of literature reproductive and developmental toxicity studies conducted with polihexanide (ECHA 2011; SCCS 2015; Johnson et al 2020). Specifically, a 2-generation study with groups of 52 rats, a 3-generation study with groups of 30 SD rats, an embryo-foetal development (EFD) study with Alderley Park rats (20 animals per group) and an EFD study with NZW rabbits (also n=20 animals per group) were conducted. In these studies, no apparent toxicity to reproduction or development (pre-, peri- and postnatal) was observed at clinically relevant exposures. In fact, NOAELs or NOAECs of reproduction and developmental toxicity endpoints in these studies were by orders of magnitude above the systemic exposure that may be expected after topical ocular administration of Akantior to *Acanthamoeba* keratitis patients. For example, a NOAEL for reproductive and offspring effects was reported in the 2-generation study at 2000 ppm, corresponding to approximately 239-249 mg/kg bodyweight/day. Similarly, in the rabbit EFD study, the developmental

NOAEL was 40 mg/kg bodyweight/day. These doses result in a much higher polihexanide exposures than will be realised in *Acanthamoeba* keratitis patients after topical ocular administration of Akantior. Even though no non-clinic and/or clinic PK study was submitted that evaluated systemic exposure of polihexanide after topical ocular administration of Akantior, it is not thought that the route of administration intended for Akantior could lead to sufficiently high systemic exposures that would exceed the exposures resulting at these NOAEL values. Considering these aspects, it is not thought that administration of Akantior to *Acanthamoeba* keratitis patients could results in a realistic risk for reproductive and developmental toxicity.

2.5.4.6. Local tolerance

In terms of local tolerance, the Applicant submitted a summary of local tolerance studies of polihexanide compiled by ECHA in 2011, and also two own studies in which the ocular tolerance of polihexanide was assessed in two *in vitro* assays.

At first, the Applicant compiled results of dermal tolerance studies. Specifically, four rabbit studies were identified, three of which were dermal tolerance studies according to the guidance provided in OECD 404. The first of these studies as well as one of the OECD 404 studies were conducted with a 20% aqueous polihexanide solution, whereas the two remaining OECD 404 studies were conducted with solid polihexanide. In the studies in which the 20% aqueous polihexanide solution was applied to the rabbit's skin, well defined to moderate erythema and slight to moderate oedema were generally observed. These reactions were, however, reversible. In the studies in which solid 96% polihexanide was applied, the skin irritating potential of polihexanide was lower than in the studies in which a 20% aqueous polihexanide solution has been applied. Furthermore, the Applicant found two literature references on ocular local tolerance, as also already compiled by ECHA in 2011. In a GLP compliant study that followed the guidance of OECD 405, and in which solid polihexanide has been applied to the eye of one rabbit, corneal opacity, vascularisation, iris inflammation and conjunctival irritation were identified. Similarly, in another study, solid 96% polihexanide powder was administered to one NZW rabbit at 0.1 g. Moderate redness and chemosis, corneal opacity, iridal congestion and corneal ulceration were observed at high severity. Altogether, these literature studies demonstrate that polihexanide imposes a serious potential for skin and especially ocular irritancy, albeit only at concentrations that are far above the expected ocular or dermal exposures after topical ocular administration of the 0.8 mg/L Akantior product to *Acanthamoeba* keratitis patients.

Additionally, the Applicant submitted two own *in vitro* ocular tolerance assays. In Study ODAK_cytotoxicity_1301, the Applicant assessed the potential toxic effects of 0.02%, 0.04%, 0.06%, and 0.08% polihexanide solutions on rabbit corneal cells (SIRC cells, Statens SerumInstitut RabbitCornea) monolayers at different exposure times (1, 5, 10, 20 and 30 minutes as well as 1, 3, and 6 hours). Cytotoxicity was then assessed by a MTT viability test system. Cytotoxicity was only observed at exposure times exceeding 10-30 minutes at the applied polihexanide concentrations (cytotoxicity-free duration depended on the polihexanide concentration). Finally, in Study ODAK-cytotoxicity_1302, the Applicant studied the cytotoxicity of polihexanide when applied to human corneal epithelium (HCE) cells in the SkinEthic™ system. Specifically, for this *in vitro* system, the Applicant described that "*the epithelial cells stratify and differentiate into a 3-dimensional tissue which bears close resemblance to normal human corneal epithelium*". In this study, polihexanide was added to the SkinEthic™ system at 0.02%, 0.04%, 0.06% or 0.08% at exposure times of up to one hour. None of these polihexanide concentrations induced relevant cytotoxicity of the HCE cells.

In their responses, the Applicant also submitted new comparative *in vitro* safety data.

Specifically, two new *in vitro* eye irritation assays were performed to evaluate whether the local safety profile of Supplier Y and a new batch of Supplier X DS (manufactured Dec. 2022) is comparable (Eurofins Study No. STULV24AA0004-1 GLP and STULV24AA0003-1 GLP; SIFI Study No. PC1301_IRRITATION_ 2401). Importantly, the Eurofins study was conducted in GLP compliance and according to OECD 492B. In Study No. STULV24AA0004-1 GLP and STULV24AA0003-1 GLP, reconstituted human cornea-like epithelium cells were treated with laboratory scale batches of polihexanide 0.8 mg/mL Supplier X and Supplier Y DS batches (tissue viability after treatment was determined by the thiazolyl blue tetrazolium (MTT) assay). Both Supplier Y and Supplier X DS at 0.8 mg/mL did not induce any irritant effect and are therefore applicable for labelling as non-irritant according to OECD 492B. Importantly, also no relevant quantitative differences in irritating potential were found between Supplier X and Supplier Y DS.

In Study No. PC1301_IRRITATION_ 2401, the Applicant treated SkinEthic™ HCE tissues with and Supplier Y and Supplier X DS at 0.8 mg/mL, and tissue viability was again examined by an MTT assay (positive and negative controls were included in this study). Also in this study, both Supplier X and Supplier Y DS did not induce any irritant effect, and also no statistically significant differences in irritation potential were found between the polihexanide batches of these two manufacturers.

However, the main caveat of these new studies is that the Supplier X batch is not the same as used during the clinical trial and therefore applicability of these data for addressing the comparability issue is severely diminished.

Of note, no *in vivo* data were generated and no batches of Supplier X that were studied in clinical trial 043 were included in this *in vitro* bridge between both DS, leaving some uncertainty regarding a possible extrapolation of *in vitro* results to the *in vivo* setup.

2.5.4.7. Other toxicity studies

Regarding antigenicity, the Applicant presented a compilation of skin sensitisation animal studies (ECHA 2011) in the original submission. Six studies with endpoint skin sensitisation were identified, in all of which 20% aqueous polihexanide solutions were administered. Specifically, two Buehler tests with guinea pigs (with group sizes of n=10) and four guinea pig maximisation tests (with group sizes varying from n=10 to n=20 in test article groups) were conducted. In the two Buehler tests, polihexanide from concentrations starting at 1% onwards was considered a moderate to even strong sensitizer. At this stage it should be remembered that the polihexanide concentration in Akantior is 0.08%. In the four guinea pig maximisation tests, the results ranged from polihexanide being a non-sensitizer to being a mild, moderate and a strong sensitizer. A concern was originally raised on the potential sensitising potency of polihexanide (see section 3.2.6 further below).

No immunotoxicity studies were submitted. However, as minimal to negligible systemic exposure of polihexanide can be expected after topical ocular administration of Akantior to *Acanthamoeba* keratitis patients, it is not thought that adverse effects to the immune system (primary and secondary immune organs as well as peripheral immune cells) are relevant.

In regards to potential dependence, no studies were submitted. However, it is not thought that polihexanide influences the release of neurotransmitters that might cause dependence. Furthermore, it is not thought that systemic polihexanide exposures after topical ocular administration of Akantior could be high enough to cause such an effect.

No metabolite studies were submitted. However, as mentioned in the non-clinical PK assessment, the Applicant reported the results of a rat biodistribution study in which radiolabelled polihexanide was used (SCCS 2015). In this study, the excreted radioactivity did not demonstrate that polihexanide was

metabolized in rats. Furthermore, it is though that systemic polihexanide exposure after topical ocular Akantior administration to *Acanthamoeba* keratitis patients will be low to negligible; potential systemic metabolism of polihexanide will therefore be quantitatively negligible.

No other toxicology studies were submitted. Of note, no information on photosafety was submitted, which however could be relevant in regards to the ocular administration of Akantior. However, in literature, polihexanide was labelled as a non-photosensitising and non-phototoxic substance (SCCS 2015).

2.5.5. Ecotoxicity/environmental risk assessment

The applicant submitted a phase I environmental risk assessment and concluded that PBT screening and PEC of Akantior do not trigger a more elaborated phase II assessment. Specifically, the Applicant communicated that the log Kow of polihexanide is -2.38 (being orders of magnitude below the trigger value of 4.5), and that the PECsw was 4.48 pg/L (also being orders of magnitude below the trigger value of 0.01 µg/L).

The applicant was asked by CHMP to amend the PECsw calculation to account for a larger drop size of 50 µl. The new PECsw value is 6.4 pg/L, and this does not alter the fact that the PECsw is well below the trigger value. Indeed, based on the very low incidence of *Acanthamoeba* keratitis in the EU (according to the Applicant the prevalence in the EU is only 5000 people), it is considered very unlikely that the use of Akantior would increase the environmental exposure of polihexanide to a relevant extent. Furthermore, as the biocidal use of polihexanide also comprises large-scale applications such as e.g. in swimming pools or in spa water treatment (Lucas 2012, DOI: 10.1007/s00128-011-0436-3), it is thought that additional environmental input of polihexanide by the use of Akantior is completely negligible. In consideration of all these aspects, it is not thought that the polihexanide content contained in Akantior could lead to adverse environmental effects.

Considering the above data, polihexanide is not expected to pose a risk to the environment.

2.5.6. Discussion on non-clinical aspects

Non-clinical pharmacology:

Several concerns were raised on the non-clinical pharmacology of polihexanide during the assessment of Akantior.

Some literature references (i.e. Narasimhan et al. 2002, Sunada et al. 2014, DOI: 10.1016/j.ophtha.2014.04.013, and Ruddell and Easty 1995) represent non-clinical studies in which the therapeutic efficacy of polihexanide against *Acanthamoeba* keratitis was either lacking, or clearly worse than comparator treatments. For example, the authors of the *in vitro* Sunada et al. 2014 study found that "*natamycin and povidone-iodine had excellent cystistatic (or cystcidal) effects, and polihexanide and propamidine did not*". Furthermore, in the study of Ruddell and Easty 1995, no significant differences in the mean grade of keratitis and in the number of culture-positive corneas between the polihexanide and the vehicle group were found in a rat model of *Acanthamoeba* keratitis. These results are summarised in the Table below:

Table 2. Results of the *in vivo* study of Ruddell and Easty 1995

Table II. Mean clinical grade of keratitis (and standard deviation) in different treatment groups according to number of days of treatment

Day	Propamidine	PHMB	Vehicle
0	2.05 (0.68)	2.20 (0.59)	2.00 (0.59)
2	1.90 (0.30)	1.95 (0.51)	1.75 (0.44)
5	1.60 (0.50)	1.80 (0.41)	1.65 (0.49)
8	1.50 (0.51)	1.65 (0.49)	1.55 (0.51)
11	1.45 (0.5)	1.70 (0.47)	1.60 (0.50)
14	1.35 (0.49)	1.30 (0.47)	1.35 (0.58)

PHMB, polyhexamethylene biguanide.

Table III. Corneal culture results according to treatment group

	Propamidine	PHMB	Vehicle
Culture-positive	5	8	9
Culture-negative	15	12	11

PHMB, polyhexamethylene biguanide.

Therefore, the Applicant was expected to:

- submit a thorough literature research in which also studies are discussed in which polihexanide displays less efficacy than comparator treatments, or in which even no efficacy was detected (of note, the study of Sunada et al. 2014 in which polihexanide showed little efficacy was not even reported in the first submission);

- thoroughly discuss the low or even lacking efficacy of polihexanide in the studies conducted by Narasimhan et al. 2002, Sunada et al. 2014 and Ruddell and Easty 1995;

- defend why polihexanide is being developed, but not treatments that showed more promising efficacy in non-clinical studies such as the ones reported in Sunada et al. 2014;

- justify why no non-clinical *in vitro* and *in vivo* studies were conducted in which the efficacy of all potential *Acanthamoeba* keratitis therapies has been compared in a relevant disease model.

In response to this concern, the Applicant provided a profound literature review of *in vitro* and *in vivo* studies in which polihexanide displayed superior, similar and inferior potential efficacy than other drugs in the treatment of *Acanthamoeba* keratitis. Out of the 10 *in vitro* studies discussed in this endeavour, the Applicant concluded that eight studies demonstrated good efficacy of polihexanide. Importantly, however, the Applicant did not perform a sufficiently broad own comparative *in vitro* and/or *in vivo* animal study with the goal of identifying which anti-amoebal agent is the most promising for further clinical development.

It is acknowledged that the overall weight of evidence in literature and in the Applicant's non-clinical studies demonstrates that polihexanide possesses anti-amoebal efficacy. However, the current literature does not allow to discriminate whether polihexanide possesses superior anti-amoebal efficacy compared to other anti-amoebal drug candidates that were commonly studied for that purpose. Importantly, some *in vitro* and *in vivo* studies demonstrated low or even lacking anti-amoebal efficacy of polihexanide. Considering these aspects, a well conducted comparative *in vitro* and *in vivo* study would have been very beneficial to discriminate which drug candidate would have been the most promising for clinical development. Without such studies, it is currently still a matter of scientific discussion about whether polihexanide falls among the more efficient anti-amoebal drug candidates or among the medium or even less efficient ones.

The conduct of an adequate non-clinical *in vitro* and *in vivo* efficacy study to answer these questions cannot be expected at later stages of the submission procedure (post D120 clock-stop). Furthermore, the efficacy of Akantior needs to ultimately be demonstrated in pivotal clinical trials. Therefore, it is concluded that this concern should be further pursued in the frame of the clinical and overall B/R assessment of Akantior.

In addition, on the basis of all the pharmacology studies presented the Applicant was asked to justify the proposed 0.8 mg/ml clinical concentration. Based on the discussion provided the Applicant has not sufficiently justified the clinical dose on the basis of the nonclinical pharmacology studies. Considering the proposed clinical dose of 0.8 mg/ml (800 µg/ml) is multiple times higher than the mean minimum cysticidal concentration (MCC) of polihexanide against *Acanthamoeba* species, efficacy should be proven based on clinical efficacy endpoints.

With regards to the Applicant's study published by Sudano Roccaro 2020, the Applicant demonstrates that variances in the molecular weight of polihexanide do apparently not alter its cysticidal activity in an *in vitro* assay, and that polihexanide batches produced by Supplier X and Supplier Y at similar molecular weight also bear the same cysticidal activity. However, of note, these two results were only gathered in an *in vitro* assay and do not necessarily display the *in vivo* situation of *Acanthamoeba* trophozoites and/or cysts in patients when facing polihexanide treatment as a result of *Acanthamoeba* keratitis. Nonetheless, on an *in vitro* level, this study is considered to potentially bridge pharmacologic efficacy between polihexanide batches of different molecular weights and different manufacturers.

Similarly, the Applicant's own Favennec 2023 study demonstrated that 0.04% and 0.06% polihexanide significantly prevented corneal lesions worsening between day 14 and 28 compared to the control group in an *Acanthamoeba* polyphagia keratitis rat model. The same efficacy was observed with 0.08% polihexanide (however, this group failed to achieve statistical significance). The polihexanide + propamidine combination treatment in this study proved less efficient in terms of clinical efficacy than the polihexanide mono-treatments. These two studies (conducted by the Applicant) therefore demonstrated good anti-amoebal efficacy of polihexanide *in vitro* and *in vivo*. Nonetheless, these studies (with the exception of the propamidine treatment in the Favennec 2023 study) did not examine whether polihexanide is superior or at least similarly effective to other frequently used anti-amoebal agents (such as e.g. chlorhexidine).

No studies on pharmacodynamic drug interactions were submitted. Principally, both additive and/or synergistic and antagonistic pharmacodynamic drug interactions are conceivable. For example, it is reported that concomitant medication is frequently used in the treatment of *Acanthamoeba* keratitis, e.g. corticosteroids are used for this purpose (Varacalli et al. 2021; DOI: 10.3390/jcm10050942). Possible implications for Akantior are discussed in more detail in the clinical assessment.

With regards to additive and/or synergistic drug-drug interactions, a multitude of non-clinical studies report beneficial effects when polihexanide is administered in combination with a second pharmacological agent. For example, literature studies demonstrate that the concomitant administration of polihexanide with a second pharmacologically active agent (e.g. cellulose synthesis inhibitors 2,6-dichlorobenzonitrile and isoxaben, Moon et al. 2015; hexamidine diisethionate, Vasseneix et al. 2006; chlorhexidine, Tirado-Angel et al 1996) can considerably increase the anti-amoebal efficacy of polihexanide. A notable example for this combinatory effect is the study published by Vasseneix et al. 2006 in which the amoebicidal activities of polihexanide, hexamidine diisethionate and miltefosine and combinations of these agents were investigated. In this study, the highest amoebicidal effect was obtained with a combination of polihexanide and hexamidine diisethionate, which was synergistic. However, polihexanide did not display any therapeutic efficacy when used as monotreatment. The results of this study are summarised in the table below:

Table 3. Results of the *in vivo* study of Vasseneix et al. 2006

TABLE 1. Time Interval of Corneal Abscess Appearance and Ratio of Rat With Corneal Abscesses on day 21 Post-*Acanthamoeba polyphaga* Inoculation

Group	Treatment	Time Interval of Corneal Abscess Appearance (Weeks, Mean + 1 SD)	Ratio of Rats With Abscesses (Day 21 PostInfection)
1	PHMB	1.6 + 0.2	6/6*
2	Hexamidine diisethionate	7.8 + 0.7	4/6*
3	PHMB + hexamidine diisethionate	1.8 + 0.1	1/6†
4	Miltefosine	1.6 + 0.3	4/6*
5	None (control)	1.8 + 0.9	5/5

Each group consisted of 6 rats.
*Not different ($P > 0.05$) or †different ($P < 0.05$) from control group and other treated groups.

With regards to the potentially bolstered efficacy of polihexanide when concomitantly administered with a second pharmacologically active agent, the Applicant was originally expected to:

- defend why no combinatory treatments with increased amoebicidal efficacy were considered for the treatment of *Acanthamoeba* keratitis;

- compile a thorough literature discussion on amoebicidal efficacies of polihexanide alone and in combination with other pharmacological agents;

- justify why no *in vitro* and *in vivo* studies were conducted to identify which mono-treatments or combination of treatments would enfold the highest amoebicidal efficacy.

The applicant acknowledged in response to this concern that the literature currently reports conflicting results about the potency of the regularly studied panel of anti-amoebal drug candidates, both alone and in combination. However, the Applicant could not provide a relevant rationale why the possibility of increased efficacy of combinatory treatments against *Acanthamoeba* keratitis was not considered and examined during the early stages of the development of Akantior. Well-conducted *in vitro* and perhaps *in vivo* studies would have provided insight into which treatments (mono or combinatory treatments) enfold the highest efficacy potential in the treatment of *Acanthamoeba* keratitis. It is likely that the results of such studies would have translated to an increased efficacy also against *Acanthamoeba* keratitis in patients. Therefore, it is likely that the Applicant developed an inferior treatment option when considering that the current state of the literature demonstrates that combinatory treatments can result in a considerable gain in efficacy.

The newly submitted comparative efficacy *in vitro* studies (especially Study No. UoB-0124-Pt1&2) and the originally submitted study by Sudano Roccaro 2020 and the DNA binding assay Study No. PC1301_PHMB-DNA BINDING ASSAY_2106 support the notion that polihexanide DS manufactured by Supplier X (manufacturing date Dec. 2022) and Supplier Y share comparable *in vitro* efficacy against *Acanthamoeba*. This is in line with the unspecific physicochemical anti-amoebal mode of action of polihexanide, being the electrostatic disruption of protozoan cell membranes and interaction with chromatin. As the molecular mode of action of polihexanide is comprised by these unspecific physicochemical interactions, it indeed appears plausible that a switch of the DS manufacturer in Akantior does not necessarily translate into relevant consequences on the efficacy of the DP. However, there still remains major uncertainty, as further outlined below.

Comparability of clinical study batches produced by Supplier X and drug batches intended for commercial use produced by Supplier Y could not be concluded on the Quality level. The provided *in vitro* studies indicated that Supplier X DS and Supplier Y DS polihexanide possess the same *in vitro* anti-amoebal and anti-cystal potency and eye irritation potential. However, it is important to anticipate that all safety and efficacy bridging studies conducted with Supplier X DS and Supplier Y DS were conducted as *in vitro* experiments. As no *in vivo* bridging studies were conducted, it is unclear whether this *in vitro* efficacy and safety bridge between Supplier X DS and Supplier Y DS can be translated to the *in vivo* complexity of *Acanthamoeba* keratitis. It is, for example, conceivable that matrix effects in the eye influence the efficacy or safety of Supplier X DS and Supplier Y DS in a different manner.

Furthermore, the comparative exercise was apparently performed with new batches from Supplier X (manufacturing date Dec. 2022) as no material from old batches (used in clinical studies) was available, despite one batch used in the phase 1 study, which was a safety and tolerability study in healthy volunteers that followed study drug administration for 14 days only. Thus, there are no data that confirm comparability of "old" Supplier X batches used for the pivotal clinical study (in patients after repeated administration and with documented efficacy and safety compared to the phase 1 study) and "new" Supplier X batches used for these new *in vitro* studies.

Based on the unspecific mechanism of action as described above, it could nonetheless be hypothesised that even apparent differences between Supplier X and Supplier Y batches may not have significant impact on clinical anti-amoebal and anti-cystal efficacy. However, this remains theoretical and uncertainty regarding the impact of differences between clinical trial material and commercial product on clinical efficacy and safety still remains. On the other hand, from available *in vitro* data it can be seen that differences in structure and/or biguanide assay do not have relevant impact on anti-amoebal and anti-cystal efficacy. Even though there is no specific comparison of clinical trial material and commercial material, these results are reassuring to some extent. Nevertheless, the remaining gap between *in vitro* data and clinical impact on safety and efficacy has been addressed on a clinical level.

Altogether, it is acknowledged that polihexanide is a well-established amoebicidal agent that has abundantly been assessed in *in vitro* and *in vivo* studies. Nonetheless, based on nonclinical literature data it appears that it is still a matter of scientific discussion whether polihexanide monotherapy is among the more promising therapies against *Acanthamoeba*, or if combinations of polihexanide with a second amoebicidal agent, or even other amoebicidal agents alone, enfold a higher therapeutic efficacy than polihexanide does.

Non-clinical pharmacokinetics:

Two concerns were raised by the CHMP on the non-clinical pharmacokinetics of polihexanide. For the first concern, the Applicant was expected to explain why no *in vivo* PK distribution studies were performed with radio-labelled polihexanide, as similar studies (albeit no ocular administration studies) were already performed in literature. The SmPC text outlines that systemic exposure to polihexanide after ophthalmic use is expected to be negligible or not to occur. In the light of this, in the second PK concern the Applicant was expected to provide a further justification of the pregnancy and breast-feeding recommendations and to discuss if more permissive recommendations may be appropriate.

In response to the first concern, the Applicant described that a PK study after ocular administration was intended, but proved not possible because no suitable analytical method for the determination of polihexanide in biological matrices could have been developed. Additionally, the Applicant elaborated why no PK study with radio-labelled polihexanide was performed. Specifically, the Applicant explained that a consistent radiolabelling of all weight fractions in the polihexanide polymer might be difficult to attain, and that therefore results of PK studies with radio-labelled polihexanide might be unreliable. This is acknowledged.

The applicant considers systemic distribution of polihexanide at its maximum daily dose after Akantior administration as negligible. Even when assuming the worst case of 100% absorption, the Applicant states that systemic exposures would nonetheless be far below published NOAELS of polihexanide as established in repeated dose toxicity studies in rats. Furthermore, the Applicant summarised literature that demonstrates that systemic absorption will be very low to negligible. This is also acknowledged.

Additionally, a new transcorneal permeation study using an ex vivo drug release test in porcine eyes in the Franz cell apparatus was performed (SIFI Study No. PC1301_EX VIVO TRANSCORNEAL PERM. STUDY_2301). In this ex vivo study, the Applicant communicates that no permeation of polihexanide and fluorescein (internal control) was detected across the porcine cornea. Importantly, it should however be mentioned that this experiment might only imperfectly represent ocular conditions during *Acanthamoeba* keratitis under which corneal barrier function might be compromised and absorption kinetics therefore enhanced.

In response to the second question, the Applicant summarised that systemic absorption after Akantior administration will be very low to negligible, and that the current state of the literature demonstrates that no direct or indirect harmful effects with respect to reproductive toxicity and no embryo-foetal toxicity were found in animal studies. However, as a precautionary measure, the Applicant did not consider more permissive recommendations in the updated version of SmPC text. This is because DART data were only taken from literature, and because no *in vivo* PK studies were performed with Akantior after topical ocular administration.

Toxicology:

The submitted repeated dose toxicity studies as well as the cited literature studies support that the planned posology of Akantior (i.e. at maximal 16 Akantior drops per day and eye containing 0.8 mg/mL polihexanide) is safe. Specifically, acute toxicity, repeated dose toxicity, genotoxicity, reproductive and developmental toxicity, local tolerance, immunotoxicity, metabolites, photosafety and environmental safety appear unproblematic at the proposed posology of Akantior. Nonetheless, some concerns were originally identified.

In regards to the antigenicity of polihexanide, it is apparent that in the two conducted guinea pig Buehler tests and in the four conducted guinea pig maximisation tests, polihexanide was mostly a skin sensitizer of moderate to even strong severity. The applicant originally did not provide a discussion on the relevance of these findings for the topical ocular administration of polihexanide to *Acanthamoeba* keratitis patients. In response to this concern, the Applicant summarised that no adverse events ascribable to sensitisation by polihexanide were reported during the clinical studies in healthy volunteers and patients with *Acanthamoeba* keratitis. Also, the Applicant communicated that in a repeated insult patch test tests conducted with 20% aqueous polihexanide (2% active ingredient) no clinically dermal sensitization was observed in the majority of participants (as reviewed by Johnson et al 2020). Considering the inconsistent results in non-clinical studies, and the important aspect that humans appear to be little sensible to sensitisation by polihexanide (e.g. also supported by Sukakul et al 2021, DOI: 10.1111/cod.13728), this particular concern was considered resolved. Importantly, patients with hypersensitivity to polihexanide are contraindicated to receive Akantior.

In the submitted rabbit repeated dose toxicity studies, the Applicant did originally not specify from which manufacturer polihexanide was obtained. Furthermore, it appears that in the submitted repeated dose toxicity studies, no concentration/formulation analyses of the administered polihexanide aliquots were conducted. In response to these concerns, the Applicant clarified that the three submitted *in vivo* studies were performed with polihexanide formulations at different concentrations which were prepared at SIFI from raw material manufactured by Supplier X. Furthermore, certificates of analysis were available of the polihexanide material used during the studies. These analyses were conducted with the UV biguanide assay. However, the Applicant specified that the different formulations were prepared by

SIFI from Supplier X material. This presumably means that the final formulations that were actually administered to the animals (e.g. after dilution by SIFI) were presumably not analysed for their polihexanide content. This renders the possibility of e.g. dilution mistakes and therefore differences of actually administered polihexanide contents versus the nominal concentrations foreseen in the experiments.

Best practice would be to analyse the API contents of all different API dosing formulations that were actually administered to the animals. However, as these measurements can obviously not be brought any more at that state of the marketing authorisation process, and as subtle changes of the actual API concentrations compared to the nominal ones would not influence the overall results gathered in the submitted *in vivo* studies, this issue is not further pursued.

Initially the Applicant calculated margins on a µg/animal and µg/kg basis comparing topical administration in rabbits to chronic oral administration in rats. Considering the Applicant claimed there is little systemic exposure this choice of calculating margins was not accepted. Now the Applicant has compared the pivotal 26 week toxicity study in rabbits to the pivotal clinical trial (Clinical Study Report 043/SI). Both studies administered polihexanide eye drops at 0.08%. This is a more appropriate method, and the safety margin of 1.35 can be accepted.

2.5.7. Conclusion on the non-clinical aspects

All non-clinical concerns were formally resolved. Regarding the *in vitro* and *in vivo* efficacy of polihexanide against *Acanthamoeba*, it appears that it is still a matter of scientific discussion whether polihexanide monotherapy is among the more promising anti-amoebal therapies, or if combinations of polihexanide with a second amoebicidal agent, or even other amoebicidal agents alone, enfold a higher therapeutic efficacy than polihexanide does. As no additional non-clinical studies can be expected during the later stages of the submission process, and as the ultimate efficacy proof of Akantior needs to be brought in pivotal clinical trials, this concern is further pursued in the clinical assessment of Akantior.

In vitro studies did not indicate relevant differences in measured effects between polihexanide products with variable analytical attributes, but no *in vivo* bridging studies were provided and it is not clear whether the described differences in both polihexanide DS on Quality level will have any consequences for the safety and efficacy on the clinical level. Furthermore, there are no data at all that confirm comparability of "old" Supplier X batches used for the pivotal clinical study and "new" Supplier X batches used for these new *in vitro* studies. The remaining uncertainty regarding the impact of differences between clinical trial material and commercial product on clinical efficacy and safety has been addressed on a clinical level (see clinical assessment for further discussion of this aspect).

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The applicant provided a statement to the effect that clinical trials were performed in accordance with GCP.

- **Tabular overview of clinical studies**

Table 4. Tabular listing of all clinical studies

Study Identifier	Location of Study Report	Objectives of the Study	Study Design and Type of Control	Test Product(s); Dosage; Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type ofReport
038/SI	5.3.5.2	Efficacy + Safety	Observational retrospective clinical study Control: NA	NA Topical/ ocular.	100	Patients affected by Acanthamoeba keratitis who were treated in 2 clinical sites from 1997 to 2007	NA	Complete; Full Report
042/SI	5.3.3.1	Safety+ Tolerability	Phase 1 (Randomised, Double-Masked, Placebo-Controlled-Multiple Dose) Control:Placebo	Polihexanide 0.4 mg/ml, Polihexanide 0.6 mg/ml, Polihexanide 0.8 mg/ml Placebo Polihexanide eye drop solution (0.4 mg/ml, 0.6 mg/ml or 0.8 mg/ml) 12 times daily (1 drop every hour during daytime) for 7 days (Days 0 to 6), followed by 6 times daily (1 drop every 2 hours during daytime) for an additional 7 days (Days 7 to 13). On Day -1, subjects received 2 test applications of the IMP, separated by 1 hour. The IMP was only applied to the right eye of all subjects. Topical/ ocular.	90 (26 patients in the 0.4 mg/ml group, 28 patients in the 0.6 mg/ml group, 27 patients in the 0.8 mg/ml group and 9 patients in the placebo group)	Healthy Subjects	14 days	Complete; Full Report
043/SI	5.3.5.1	Efficacy + Safety + Tolerability	Randomized, Assessor-Masked, Active-Controlled, Phase 3 Study Active Comparator: 0.2 mg/ml Polihexanide + 1 mg/ml propamidine combination therapy	0.8 mg/ml Polihexanide + placebo 0.2 mg/ml Polihexanide + 1% propamidine combination therapy 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 Topical/ ocular.	130 planned, 135 randomized 134 analyzed (69 subjects received 0.8 mg/mg Polihexanide + placebo; 65 subjects received 0.2 mg/ml Polihexanide + 1 mg/ml propamidine)	Subjects affected by Acanthamoeba keratitis.	Until resolution in the affected eye up to a maximum of 12 months.	Complete; Full Report

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

No dedicated clinical pharmacokinetic measures have been conducted in the clinical developmental programme for Akantior.

Pharmacokinetic studies in humans have established that polihexanide is poorly absorbed through intact human skin and, when administered systemically, it is completely and rapidly cleared from the body by excretion in the urine (Shah 2004). Oral administration of radiolabelled polihexanide estimated absorption at ≤7%. Only low molecular weight polihexanide (1230 to 1235 Daltons) is absorbed from the intestine and excreted intact in urine (Shah 2004).

Polihexanide is intended for topical application to the eye and is not expected to reach significant levels in the blood. Even in the case of 100% absorption, each 0.08% polihexanide eye drop containing 40 µg polihexanide would give a blood concentration of 8 ng/ml. This is approximately 250- to 1000-fold lower than its cytotoxic concentration for *in vitro* corneal keratocytes (Lee 2007).

After 12 topical instillations of 0.08% polihexanide eye drops, the highest potential polihexanide blood concentration obtainable would be approximately 90 ng/ml, 80 times below the highest polihexanide concentration (7.5 µg/ml) obtainable assuming complete absorption of the maximum daily tolerable oral intake in humans (41.1 mg, SSC 2015).

CHMP advice from June 2016 agreed that plasma polihexanide levels would be likely to be far below a clinically-relevant level when administered using the polihexanide ophthalmic dosing regimen used in the Phase I and III clinical studies. As a result the availability of data on plasma polihexanide levels was deemed non-mandatory from the regulatory perspective.

According to the clinical study protocol (dated Dec 04, 2015), PK measurements of the polihexanide bioavailability in plasma were planned to be performed. The day after each administration schedule (Days 7 and 14), a 2 mL blood sample was taken from each subject for the analysis of polihexanide in plasma. The exact time point of blood sampling was recorded in the eCRF, as was the time and date of the last eye drop administered. However, polihexanide concentrations in plasma were not determined because the levels were likely to be far below a clinically relevant level and it would be impossible to obtain reliable PK data from such measurements. As it would be impossible to make an adequate determination of polihexanide levels in plasma, the CHMP agreed that this was not mandatory from a regulatory point of view. Therefore, pharmacokinetic data were not included in the clinical study report.

Pharmacokinetic interaction studies

No interaction studies have been performed.

2.6.2.2. Pharmacodynamics

No clinical pharmacodynamic (PD) or pharmacokinetic-pharmacodynamic (PK/PD) study was conducted.

Mechanism of action

Polihexanide is active against *Acanthamoeba*, not only in the active trophozoite form but crucially also in the dormant cyst form; its dual targeted mechanism of action involves:

• Disruption of *Acanthamoeba* cell membranes. Polihexanide binds to the phospholipid bilayer of the trophozoite membrane causing membrane damage, cell lysis and death due to leakage of essential cell components. Crucially, polihexanide is also able to penetrate the ostiole into the encysted organism to exert the same effect (Khunkitti,W et al, 1996; Khunkitti, W et al, 1998, Seal D et al, 2003; Firdessa R et al, 2015; Horner I.J et al, 2015)

• DNA binding. Once it has passed through the membrane, polihexanide condenses and damages *Acanthamoeba* chromosomes. It interacts extensively with the DNA phosphate backbone to block *Acanthamoeba* DNA replication (Firdessa R et al, 2015; Chindera K et al, 2016; Sowlati-Hasjin S et al, 2020).

In contrast to most antimicrobials, resistance to polihexanide is rare due to its mechanism of action.

2.6.3. Discussion on clinical pharmacology

No dedicated clinical pharmacokinetic study was conducted and no PK evaluation was done in the submitted studies to determine polihexanide concentrations. Polihexanide is intended for ocular use as eye drops. The applicant argues that plasma levels following ophthalmic dosing are estimated to be far below clinically relevant levels. During scientific advice in 2016 (EMA/CHMP/SAWP/401053/2016), the Applicant argued that detection of polihexanide in biological samples is not achievable in sufficient quality based on available analytical methods, which compromises concentration determination in non-clinical and clinical studies. This argumentation was followed by the CHMP in this scientific advice procedure. Thus, neither local ocular, nor systemic concentrations were determined for polihexanide after ocular use in non-clinical or clinical studies. However, as discussed in the non-clinical section in more detail, exposure determination appears principally possible at least in the non-clinical study setting. For the clinical setting, it is understood that ocular exposure would have to be determined with

invasive ocular procedures, that carry more risk than the drug application (eye drops) itself. Thus, the lack of clinical ocular exposure data can be followed. However, the lack of non-clinical or clinical data on exposure (ocular and systemic) is critically noted, as it compromises the Applicant's hypothesis that the bioavailability of polihexanide in the corneal stroma is likely to be increased by increasing the concentration of polihexanide above 0.2 mg/ml. Still the Applicant concluded that the use of polihexanide 0.8 mg/ml can be expected to reduce the treatment failures seen with polihexanide 0.2 mg/ml. In fact, worsening of disease was only observed in the treatment group treated with 0.8 mg/ml polihexanide, but not for subjects treated with the combination of 0.2 mg/ml polihexanide and 1 mg/ml propamidine (even though prior steroid use might be the driving factor for this observation, as 3 of 4 subjects had used prior steroids, see clinical efficacy section below). This indicates some substantial uncertainty regarding exposure levels and clinical outcomes. The lack of non-clinical pharmacokinetics is critically noted in this aspect as also the hypothesis that higher concentrations of polihexanide penetrate deeper into the cornea was not substantiated by the Applicant (see discussion on clinical efficacy for more details). Available non-clinical data regarding systemic toxicity do not give rise to concern. Similarly, clinical safety data do not indicate systemic exposure in safety relevant levels (see clinical safety section below).

No dedicated clinical studies to assess the pharmacodynamics of polihexanide were conducted. The applicant argues that studies were not applicable due to its mechanism of action as an antimicrobial agent. Indeed, clinical studies in healthy subjects do not appear relevant and would not provide conclusive evidence for this therapy in patients with *Acanthamoeba* keratitis, and patient numbers are very limited due to the rarity of the disease. It is agreed that the effect of polihexanide on *Acanthamoeba* cellular membranes is well described in literature and also the mechanism regarding DNA binding and chromosome condensation as proposed appears confirmed (see non-clinical AR for a more detailed discussion on described mechanism(s).

No clinical or non-clinical studies on pharmacodynamic drug-drug interactions were submitted on . Principally, additive and/or synergistic and antagonistic pharmacodynamic drug interactions are conceivable. It is reported that concomitant medication is being used in the treatment of *Acanthamoeba* keratitis. For example, corticosteroids are used for this purpose (Varacalli et al., 2021; DOI: 10.3390/jcm10050942). No thorough discussion about the concomitant use and local interaction potential of Akantior towards other administered ophthalmic medications (especially steroids and IOP lowering drugs) was provided, but the Applicant claims that interactions are very unlikely in microbial keratitis treatments. Still, it remains uncertain whether potential local interactions (e.g. on physicochemical level) might occur, and thus a statement to inform that no interaction studies have been performed was inserted in section 4.5 of the SmPC. The uncertainty appears sufficiently addressed by informing the intended users. Also, the instruction for a 5-minute interval between administrations of ophthalmic products outlined in the PI is acknowledged. It is agreed that systemic interactions are not expected, as systemic exposure is negligible.

2.6.4. Conclusions on clinical pharmacology

In conclusion, the claim that increasing concentrations of polihexanide increase concentrations in the corneal stroma and lead to a reduction in treatment failures cannot be followed as no support for this hypothesis was provided. The lack of systemic safety findings (at a non-clinical and clinical level) are reassuring regarding possible systemic exposure.

It is considered acceptable that no clinical PD studies have been conducted, considering literature data and provided non-clinical information (i.e. *in vitro* dose response and *in vivo* dose toxicity studies).

2.6.5. Clinical efficacy

2.6.5.1. Dose response study(ies)

ODAK Phase I (042/SI): Randomised, Double-Masked, Placebo-Controlled, Multiple-Dose Phase I Study to Evaluate the Safety and Tolerability of Different Doses of Preservative-free Polyhexamethylene Biguanide (polihexanide) Ophthalmic Solution in Healthy Subjects.

Polihexanide eye drop solution (0.04%, 0.06% or 0.08%) 12 times daily (1 drop every hour during daytime) for 7 days (Days 0 to 6), followed by 6 times daily (1 drop every 2 hours during daytime) for an additional 7 days (Days 7 to 13). On Day -1, subjects received 2 test applications of the IMP, separated by 1 hour.

Study objective: The objective of the study was to establish the ocular safety and tolerability, and systemic safety, of 3 different doses of preservative-free polihexanide in healthy subjects. Safety and tolerability of the dose groups (0.04% polihexanide with n=26, 0.06% polihexanide with n=28 and 0.08% polihexanide with n=27) was compared to placebo (n=9).

The primary endpoint was the rate of dose limiting events (DLEs) in each dose group leading to premature withdrawal of IMP, including severe life-threatening or blinding events (serious adverse events [SAEs]).

Polihexanide concentrations in plasma were not determined because the levels were likely to be far below a clinically relevant level and it would be impossible to obtain reliable PK data from such measurements. As it would be impossible to make an adequate determination of polihexanide levels in plasma, the CHMP at EMA agreed that this was not mandatory from a regulatory point of view.

Results for this phase I study only pertain safety aspects and are thus presented in the Safety section of this report.

2.6.5.2. Main study

Study 043/SI ODAK Phase III

Study 043 was a randomised, assessor-masked, active-controlled, phase III study to evaluate efficacy, safety and tolerability of 0.08% polyhexamethylene biguanide (PHMB, polihexanide) ophthalmic solution in comparison with 0.02% polihexanide + 0.1% propamidine combination therapy in subjects affected by *Acanthamoeba* keratitis.

Methods

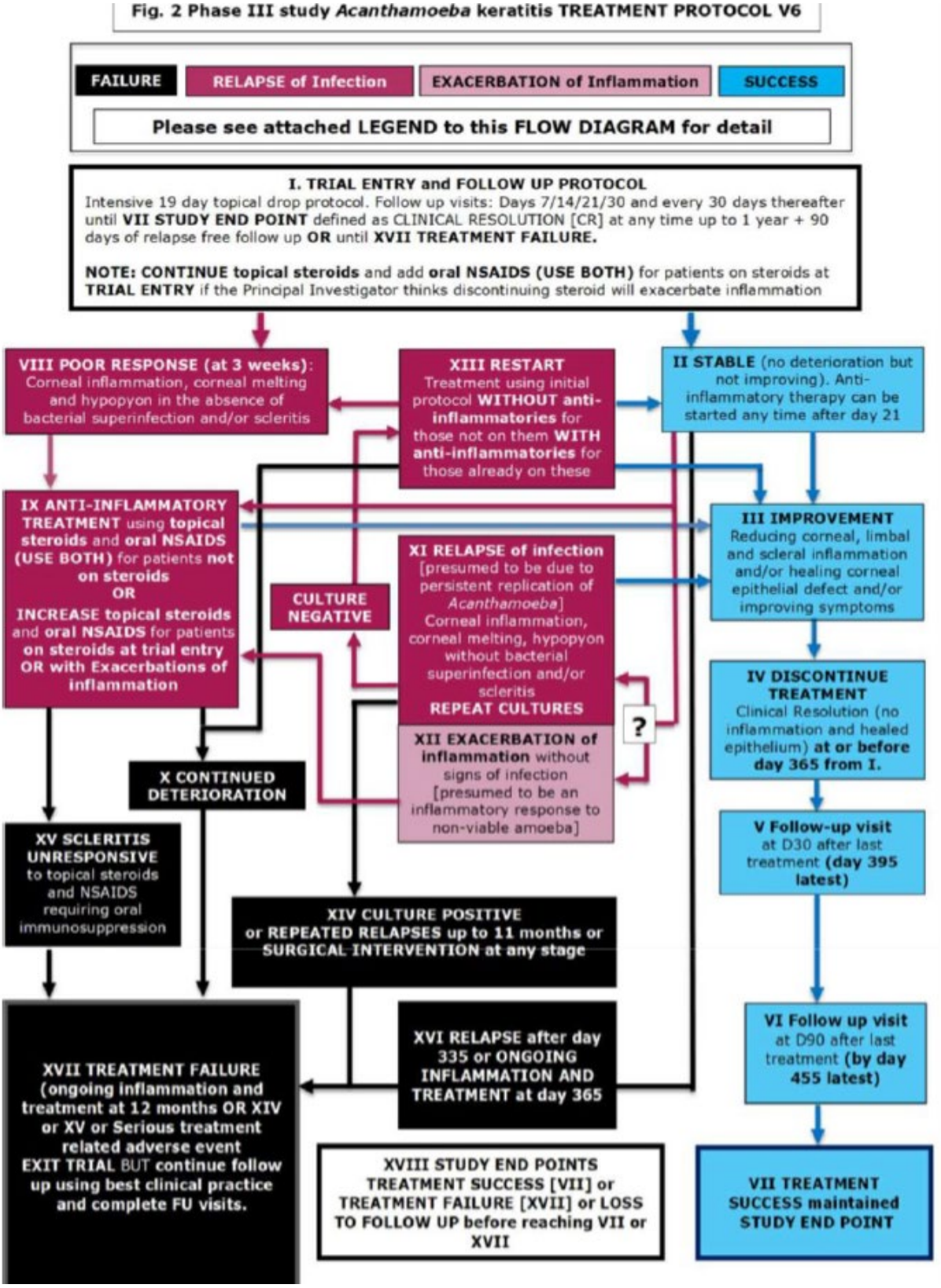
Study 043/SI (also referred to as study 043) was a randomised, double-masked, double-dummy, active-controlled, multi-centre, parallel-group Phase III study to evaluate the efficacy, safety, and tolerability of 0.8 mg/ml polihexanide ophthalmic solution compared to the active comparator (0.2 mg/ml polihexanide + 1 mg/ml propamidine combination therapy) in male and female subjects affected by *Acanthamoeba* keratitis.

The study was designed as a superiority study with the possibility to test for noninferiority if the superiority hypothesis was not met, according to the requirements of the guidance from the European Agency for the Evaluation of Medicinal Products (EMA) (CPMP/EWP/482/99).

A total of 130 subjects affected by *Acanthamoeba* keratitis were planned to be assigned on a 1:1 basis to receive either:

- Group 1: 0.8 mg/ml polihexanide + placebo
- Group 2: 0.2 mg/ml polihexanide + 1 mg/ml propamidine combination therapy.

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.

- **Study Participants**

Inclusion Criteria

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

1. Subject must have been able and willing to give informed consent.

2. Male or female subjects of any race must have been ≥12 years of age, inclusive. subjects <18 years were only enrolled in selected study sites.

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

4. Clinical findings had to be consistent with *Acanthamoeba* keratitis.

Clinical findings included 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 had to be consistent with *Acanthamoeba* keratitis (performed within 7 days prior to study entry or as part of screening procedures)

• Confocal microscopy findings included: 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 were eligible for the study:

• Antibiotics: subjects who had an ocular bacterial infection at baseline were eligible for the study. However, only topical moxifloxacin was permitted, unless resistant or contraindicated.

Note: subjects who developed intercurrent bacterial infections were 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 polihexanide or Chlorhexidine) for a misdiagnosis could be included, but must have discontinued these drugs after entry into the study.

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

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

7. Females of childbearing potential were included if they were 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); bilateral tubal occlusion.

Sexual abstinence was 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 was only acceptable if in line with subjects preferred and usual lifestyle.

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

8. Females of childbearing potential had to 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 had to agree 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 had to agree to comply with inclusion 7 or 8. For a vasectomized male who had his vasectomy 6 months or more prior to study start, it was required that they use a condom during sexual intercourse. A male who had been vasectomized less than 6 months prior to study start must have followed the same restrictions as a non-vasectomized male.

Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and LAM were 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.

Exclusion Criteria

Individuals who met any of the following criteria were not eligible to participate 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 (polihexanide, 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 was 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 have ensured that all study enrolment criteria were met at randomisation. If a subject's status changed after randomisation, but before the first dose of study drug was given, such that he or she no longer met all eligibility criteria, then the subject should have been excluded from participation in the study.

In case of bilateral infection, only the right eye (or the worst affected eye) was included in the study. The fellow eye was treated with the best treatment according to clinical practice.

- **Treatments**

A total of 130 subjects affected by *Acanthamoeba* keratitis were planned to be assigned on a 1:1 basis to receive either:

• Group 1: 0.8 mg/ml polihexanide + placebo

• Group 2: 0.2 mg/ml polihexanide + 1 mg/ml propamidine combination therapy.

Dose regimen

The first application of study medication was performed at the research centre (after completion of all baseline assessments and randomisation) and subjects received instructions on how to apply the eye drops in the affected eye. Thereafter, subjects left the clinical research centre and study drug was self-administered at home. When the subject is at the clinical research centre for assessments, subjects applied the study drug themselves at the clinical research centre.

Table 5. Dosing Schedule

Day	Dose
0 to 5	1-hourly drops (16 drops in a day) for 5 days
6 to 12	2-hourly drops (8 drops in a day) for 7 days
13 to 19	3-hourly drops (6 drops in a day) for 7 days
20 onwards	4 drops in a day until resolution (maximum 12 months)

On Days 0 to 5, subjects applied study drug every hour daytime only (1 drop of each ophthalmic solution in the affected eye). On Days 6 to 12, (1 week), subjects applied study drug every 2 hours daytime only. On Days 13 to 19, (1 week), subjects applied study drug every 3 hours daytime only. On Day 20 until resolution, subjects were to apply study drug 4 times a day at daytime only. Subjects were to be treated for the maximum of 1 year after randomisation. For both treatment groups (0.8 mg/ml polihexanide + placebo), and (0.2 mg/ml polihexanide + propamidine), dosing followed the same protocol.

The control therapy with patients receiving 0.2 mg/ml polihexanide + 1 mg/ml propamidine combination therapy is chosen as the most widely used alternative in the EU. Regarding the frequency of application, the most commonly used and described treatment protocol is that used at Moorfields Eye Hospital London (Dart et al., 2009) comprising an initial intensive regimen followed by a less frequent dosing.

Adjunctive therapy

Adjunctive therapy was defined as new use after baseline or any increase of at least one of following medication with ATC5: Topical steroid: dexamethasone, prednisolone, fluorometholone, loteprednol Oral non-steroidal anti-inflammatories (NSAIDS): diclofenac, ibuprofen, flurbiprofen, naproxen.

Concomitant Medications

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

Antibiotics: Topical moxifloxacin was permitted for the treatment of intercurrent bacterial infections (unless culture and sensitivity, or clinical progress demands a change). Topical moxifloxacin was not permitted for use as a prophylactic antibiotic in subjects with corneal ulcers; polihexanide 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 was not allowed during the study. Subjects using antiviral or antifungal drugs at study entry, had to have those discontinued.

Anti-inflammatory drugs: For subjects using topical steroids at study entry, there were the following options:

a. Stop steroids, OR maintain, OR reduce doses (at the investigator's discretion). Unpreserved dexamethasone (0.1% or 0.15%) was the only topical steroid permitted for use in this trial. Subjects using any other topical steroids on trial entry were to be changed to this at the appropriate frequency. Diclofenac was the only oral NSAID permitted in the trial and was to 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 were used during the study.

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

c. Subjects using no topical steroids at study entry could have had 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 were permitted.

Treatment adherence

Subjects who missed 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 were not discontinued but were classified as non-adherent. Subjects were discontinued from the study if, in the opinion of the investigator, drug regimen adherence was insufficient.

- **Objectives**

-Primary Objective

The primary objective of this study was to compare the Clinical Resolution Rate (CRR) at 12 months from randomisation (CRR_12) of 0.8 mg/ml polihexanide + placebo with that of 0.2 mg/ml polihexanide + 1 mg/ml 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 0.8 mg/ml polihexanide monotherapy.

-Secondary Objectives

The secondary objective of this study was to obtain additional safety information on 0.8 mg/ml polihexanide ophthalmic solution.

-Hypotheses

The study was designed as a superiority study with the possibility to test for non-inferiority if the superiority hypothesis was not met.

The primary hypothesis to be tested is that the CRR_12 of subjects treated with 0.8 mg/ml polihexanide 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 mg/ml polihexanide + 1 mg/ml propamidine combination therapy.

Null hypothesis for superiority testing:

• No difference between test treatment and reference treatment

Null hypothesis for non-inferiority testing:

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

Secondary hypotheses are:

• That adverse events, and those relating to toxicity in particular, are less with polihexanide 0.8 mg/ml monotherapy compared to the comparator.

• That time to a cure is shorter in subjects receiving polihexanide 0.8 mg/ml monotherapy compared to the comparator.

Note: The estimated CRR_12 from the start of treatment is 67% for the active comparator 0.2 mg/ml polihexanide + 1 mg/ml 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).

-Justification for the choice of the non-inferiority margin

On statistical grounds

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

Table 6. Selection of Non-inferiority Margin (Δ) based on statistical issues

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

On clinical grounds

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

• A poor response to therapy is common in this disease. Disease progression is slow in this disease (over weeks and months) and clinicians have to assess the effect of treatments every 1-2 weeks, in cases not progressing well, and modify therapies to optimize outcomes. Because of the disease chronicity the use of an ineffective treatment for these periods results in a delayed response, but no serious short term morbidity. The study results may indicate non-inferiority, when at worst the true difference in proportion 'cured' (Combined - polihexanide alone) is 0.20. The most likely impact of such a finding on clinical practice will be to encourage the use of polihexanide monotherapy as a first line treatment.

• However, if polihexanide monotherapy fails in clinical practice during the early stages of treatment, which is to be expected in some subjects, the clinician is unlikely to abandon the good clinical practice of monitoring the disease progress closely and adding other antiamoebics to the therapy when necessary.

• At worst, the impact of the study finding might result in a little delay (matter of days) in starting a combined treatment in some patients, whilst giving a chance for the biguanide monotherapy to act. This scenario is unlikely to result in blindness or serious morbidity. It may cause some delay in the clinical resolution.

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

The Study 043/SI protocol included all conditions specified by the CPMP/EWP/482/99 guidance, to meet the strict requirements of a non-inferiority study; this made it feasible to test for non-inferiority using the results of the superiority analysis and the approach was agreed with the EMA (EMA/CHMP/SAWP/429512/2014; EMA/CHMP/SAWP/401053/2016). The conditions specified:

1) a pre-defined non-inferiority margin based on both clinical and statistical considerations.

2) comparable results in the ITT and PP populations with respect to p-values and confidence intervals.

3) a study design that aimed to minimize protocol deviations; and

4) evidence that the best supportive care used as comparator (i.e., combination of 0.2mg/mL polihexanide + 1.0 mg/mL propamidine) showed its expected level of efficacy.

- **Outcomes/endpoints**

-Primary efficacy variable

The primary efficacy variable chosen to assess drug efficacy is the CRR_12 (the clinical resolution rate at 12 months from randomisation, defined as the percentage of subjects cured 30 days after discontinuing all study therapies, within 12 months of randomisation).

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 hyperaemia): mild conjunctival inflammation is acceptable if related to other concurrent conditions such as blepharitis.
- No limbitis, scleritis or anterior chamber inflammation.
- No relapse within 30 days of discontinuing all topical and systemic therapy given for *Acanthamoeba* keratitis.

For regulatory and verification reasons, there is an additional 60-day follow-up to exclude late relapses. Relapse of infection is identified by a positive culture, unfortunately very insensitive due to the persistence of deep organisms, supported by an increase in cysts on confocal (also insensitive in severe disease) or using clinical criteria: development of more severe corneal inflammation, melting, ulceration hypopyon, development of ring abscess necessitating another intensive course of therapy.

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

-Secondary efficacy variables

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

The other secondary efficacy variables are:

- *BCVA*

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

- *Pupil test (swinging light test)*

  A pupil test (swinging light test also known as a test for a relative afferent pupillary defect) to detect retinal or optic nerve involvement when the retina cannot be examined due to the presence of corneal opacity or cataract. Pupil test is reported as Normal; Abnormal, NCS; Abnormal CS. The specific abnormalities are listed.

- *Corneal scarring as identified by slit lamp examination*

  Corneal scarring is being assessed as Present or Absent, using the slit lamp biomicroscopy performed by the examining ophthalmologist.

- *Ulceration severity* as identified by slit lamp examination using a 2-grade scoring procedure

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

- *Anterior chamber inflammation* as identified by ophthalmoscopy using a 3-grade scoring procedure

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

- *EQ-5D questionnaire and VFQ25 questionnaire*

  EQ-5D-5L and VFQ5 are two instruments for subject-reported outcomes; the former addressing the overall health status of the subjects and the latter their visual function status. The EQ-5D is a quality of life instrument consisting of a self-description of health state in term of five dimensions (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression) using a five-level scale, score 1 being the best and score 5 the worst. In addition, EQ-5D comprises an evaluation component in which a patient records their overall health state using a visual analogue scale (VAS) which can capture problems that are not captured within the 5 dimensions of EQ-5D. The VFQ-25 is a 25-item questionnaire assessing the effect of visual

impairment on quality of life. The sum of responses is the composite score. This score was recoded for statistical analysis with score 100 being the best and score 0 the worst.

- **Sample size**

A total of 130 subjects affected by *Acanthamoeba* keratitis were to be assigned to one of the 0.8 mg/ml polihexanide monotherapy and 0.2 mg/ml polihexanide + 1 mg/ml propamidine combination therapy in a ratio of 1:1.

From the results of the Sponsor's observational, case series retrospective study 038/SI, a CRR_12 of 67% for the active comparator of 0.2 mg/ml polihexanide + 1 mg/ml propamidine was expected. This figure is in the range of cure rates described in the literature. If the true difference in CRR_12 is 0.20 (Δ) (or more) in favour of polihexanide monotherapy (0.8 mg/ml polihexanide), 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 would be reduced from 67% to 63%. To account for the inclusion of this group, the sample size was adjusted to 130 subjects (allowing for 10% loss to follow-up, i.e. 116 evaluable subjects).

- **Randomisation and Blinding (masking)**

Randomisation was 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) were evenly balanced across treatment groups, and to enhance the validity of comparisons across treatment groups.

Subjects were assigned to either Group 1 or Group 2 using a 1:1 randomisation schedule. The randomisation schedule was generated using a computer programme and verified for accuracy using strict quality control procedures.

Eligible subjects received a masked treatment assignment with a unique randomisation code based on the randomisation list. The assigned randomisation code was captured in the electronic CRF. This randomisation code did not disclose any treatment assignment.

Only one eye was included in the study and randomised to the study treatment. The worst affected eye was selected as the study eye unless both eyes were equally affected when the right eye was selected. The non-study eye was treated with the best treatment according to clinical practice at each study centre.

The study is double-masked to reduce potential bias during data collection and evaluation of endpoints. Patients received a masked treatment assignment with a unique randomisation code based on the randomisation list. The code was allocated by the pharmacy and patients were randomised when they collected their first study drug kit from the pharmacy. Since each subject received two different 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 used single-use vials (0.8 mg/ml polihexanide or 0.2 mg/ml polihexanide) and multi-use containers (placebo or 1 mg/ml propamidine).

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 treatment assignment for

subjects continuing to participate in the study. To reduce this risk, and to protect the integrity of the data, efficacy assessments were 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 were not seen by the doctors carrying out the study. The investigator and other study staff, the subjects, the monitors and the sponsor had only access to masked treatment assignment information on a 'need to know basis' until data collection had been completed, the database was locked and the protocol deviations and the primary reason for discontinuation from the study for each subject were determined. Until unmasking, treatment assignment information was only accessible to qualified Sponsor personnel responsible for pharmacovigilance reporting. Emergency unmasking was possible only if considered relevant for medical care of the patient with consequent discontinuation from the study. The randomisation code was broken when all patients had finished the study.

The randomisation code does not disclose any treatment information. Until the moment of unmasking, the treatment assignments linked to the codes were accessible only by the Qualified Person responsible for Pharmacovigilance of the sponsor. Emergency Unmasking was only done if knowledge of treatment assignment is considered relevant for medical care of the patient. In other cases, the randomisation code will be broken when all subjects have finished the study.

No subjects were unmasked during the course of the study.

- **Statistical methods**

-Analysis sets

Full Analysis Set (FAS): All subjects who were randomised and for whom the diagnosis of *Acanthamoeba* keratitis was confirmed and the primary efficacy variable was assessed. The FAS is used in all efficacy analyses. Analysis using the FAS will be based on the planned treatment (i.e. subjects will be analysed 'as randomised').

Per Protocol Analysis Set (PPAS): The subset of subjects in the FAS for whom no major protocol deviation judged as having an impact on the primary efficacy analysis was reported or identified. Subjects assigned to the PPAS attended the 30- and 90-day follow-up visits. The last disease status assessment within 12 months of randomisation was used for disease status assessment. For subjects with disease resolution at that last visit, the 30-day and 90-day follow-up assessments should confirm disease resolution. Otherwise, the disease was regarded as not having been resolved. The decision as to which protocol deviations should be considered as reason for exclusion from the PPAS should be made at the clean file meeting and documented in the clean file report. Analysis on the PPAS will be based on the actual treatment (i.e. subjects will be analysed 'as treated'). The primary efficacy variable, CRR_12, will also be evaluated using the PPAS.

Safety Analysis Set (SS): All subjects who received at least one dose of study medication are included in the Safety Analysis Set. This set is used in all analyses of safety data.

The Full Analysis Set [FAS] was used in all efficacy analyses. The PPAS was used in the analysis of the primary efficacy variable only.

-Statistical analyses

<u>Primary efficacy analysis</u>

The main endpoint disease resolution is assessed at each visit up to study day 365. Clinical resolution 12 months after baseline for a patient is defined as the percentage of subjects cured 30 days after discontinuing all study therapies, within 12 months of randomisation. There should be no relapses after (the last) clinical resolution classification. Discontinued subjects will be regarded as not cured.

It is also possible to test for non-inferiority if the superiority hypothesis is not met. The 90% CI for the difference between the treatments obtained from the superiority analysis gives the necessary information. Position of the lower end of the CI relative to a pre-defined agreed 0.20 non-inferiority margin (Δ) provides the key information for making decisions (conclusions) about non-inferiority.

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

Secondary efficacy analyses

Time-to-cure

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

BCVA

The VAMS states that: "Depending on the problem, this notation can be most useful when analysing or graphically plotting visual acuity scores because equal linear steps on the LogMAR scale represent equal ratios in the standard size sequence." Hence, BCVA is presented and analysed using LogMAR values. ANCOVA is used with treatment and the Baseline value of LogMAR as predictors. Refraction at Baseline may also be used as a predictor.

EQ-5D-5L3 & EQ VAS, at all visits

EQ-5D-5L consists of 5 dimensions, Mobility, Self-care, Usual activities, Pain/discomfort, and Anxiety/depression, each answered with one of five different answers. The EQ-5D is summarised for each dimension. A table presenting the number of subjects reporting a problem, i.e. a score higher than 1, will also be presented. A repeated measures ANCOVA for each dimension is conducted using treatment and the baseline value as predictors.

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

Corneal scarring/Ulceration severity

Corneal scarring/Ulceration severity at EoT is analysed with logistic regression using treatment group and corneal scarring/ulceration severity at Baseline at baseline as predictors.

Anterior chamber inflammation

The anterior chamber inflammation 4-grade scores is analysed using an ordinal logistic regression approach with treatment and the Baseline anterior chamber inflammation as predictors.

VFQ-25

The Visual Functioning Questionnaire consists of 25 questions, two with one or three sub-questions designed to elucidate the answer to the main question. Averaging the means for each scale for each patient, excluding the General health score, provides a composite score for each patient, which can then be used to calculate the composite score for each treatment group.

In general, logistic regression was used for binary variables, a proportional odds model was used for an ordinary scaled variable, an analysis of variance (ANOVA) or covariance (ANCOVA) was used for continuous variables as appropriate. If the underlying assumptions were not fulfilled, data transformation or a nonparametric test was to be performed. For comparison of proportions, Chi square tests, Fisher's exact test, or Mantel-Haenszel procedures, as appropriate, were considered. For continuous variables, the Wilcoxon rank-sum test or rank ANOVA/ANCOVA were used.

-Multiplicity

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

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

Hypotheses tested for other secondary variables are analysed using significance levels of 0.05.

Subgroup analyses

No subgroup analysis was pre-defined. Subgroup analyses by risk factors (staging of *Acanthamoeba* keratitis at baseline, prior steroid treatment) were performed.

Staging of *Acanthamoeba* keratitis at baseline:

Stage 1 AK: is defined as the presence of corneal epitheliopathy only.

Stage 2 AK: is defined by the presence of = 1 corneal epithelial defects, perineural infiltrate or stromal infiltrate in addition to stage 1 findings.

Stage 3 AK: require the presence of a corneal ring infiltrate and 1 or more features of stage 2 disease.

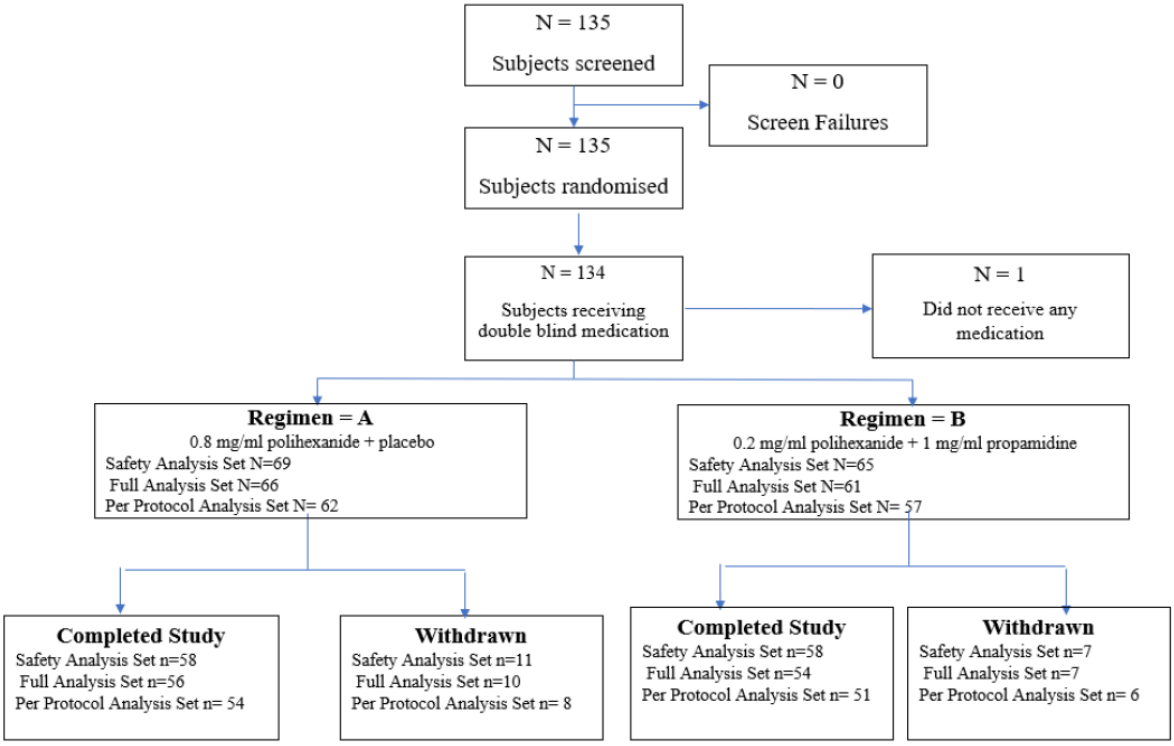
Subjects who were taking antiviral, antifungal, antibiotic, and steroid drugs prior to their participation in the study were considered or categorized as subjects with risk factors.

Results

- **Participant flow**

Subject Disposition

Figure 3. Diagram for subject disposition



Protocol deviations

A total of 8 subjects reported the major protocol deviations in the study 043/SI and were not included in the PPAS. Four subjects in monotherapy 0.8 mg/ml polihexanide + placebo and 4 subjects in the combination therapy 0.2 mg/ml polihexanide + 1 mg/ml propamidine. These major deviations were related to the use of forbidden medications (n=3) or lack of adherence to the dosing schedule (n=4) or protocol procedures (n=1).

Table 7. Major Protocol Deviations in patients with *Acanthamoeba* keratitis

Unique subject identifier	Treatment	Protocol Deviation Term
ODAK-043-11-11	Comparator	Subject used Brolene prior to enrolment which is a violation of inclusion criterion 2
ODAK-043-11-14	Test	Subject used Brolene prior to enrolment which is a violation of inclusion criterion 2.
ODAK-043-12-01	Test	It seems that the subject did not use the IMP according protocol. No corrective action is possible for this subject

ODAK-043-12-02	Test	2nd scraping shows HPV infection. Patient went off study immediately
ODAK-043-13-01	Comparator	Pharmacy uses incorrect dosing instruction labels.
ODAK-043-21-37	Comparator	Patient had anti-viral medication to treat herpetic keratitis on the day that he stopped the study medication due to having obtained Clinical Resolution.
ODAK-043-22-03	Test	Culture not done when symptoms worsened
ODAK-043-22-10	Comparator	Subject did not return the 1st Continuation kit at month 4. The 2nd Continuation kit was correctly dispensed at month 4. Subject discontinued the study after 1 month and 1 week after the dispense of…?
ODAK-043-22-15	Test	IMP non-compliance throughout study. Site discussed with patient who reported that he did not use eye drops as prescribed because he was not seeing results.

- **Recruitment**

This was a multicentre study and patients were recruited in 6 centres (3 centres in the UK, 2 centres in Italy and 1 centre in Poland).

First patient, First Visit: 17 August 2017

Last patient, Last Visit: 18 June 2021

- **Conduct of the study**

Protocol compliance

The final Statistical Analysis Plan is dated 01 July 2021 (Final version 3) and a Data Review Meeting took place on the same day. The blind was broken after the Database lock on 08 October 2021.

The original Protocol (dated 25 January 2017) was amended 1 time. The rationale for the update (dated 08 October 2018) was to incorporate significant changes: treatment of both eyes, corrections of number of visits after relapse, and removal of 0.8 mg/ml polihexanide treatment after the end of the study. No changes to the planned analyses have been considered. However, the definition of the PPAS have been altered compared to the protocol due to one wrong sentence.

The use of normal-distribution test assumptions was the primary choice as described in Section 7.11 in the SAP v 3.0, 01-Jul-2021. If such assumptions were found incorrect during the analysis of the data, an assessment of which type of analysis to be used was conducted.

GCP inspection

A national GCP inspection was conducted at one study site in Poland, which did not give rise to major concerns.

- **Baseline data**

Table 8. AK stage by treatment group at baseline (FAS)

AK stage	0.8 mg/ml polihexanide + placebo	0.2 mg/ml polihexanide + 1 mg/ml propamidine	All subjects N= 127 n (%)
Stage 1*	14 (21.2%)	8 (13.1%)	22 (17.3%)
Stage 2**	41 (62.1%)	46 (75.4%)	87 (68.5%)
Stage 3***	11 (16.7%)	7 (11.5%)	18 (14.2%)
Total	66 (100%)	61 (100%)	127 (100%)

AK=*Acanthamoeba* keratitis
*Stage 1 AK: was defined as the presence of corneal epitheliopathy only.
**Stage 2 AK: was defined by the presence of ≥1 corneal epithelial defects, perineural infiltrate or stromal infiltrate in addition to stage 1 findings.
***Stage 3 AK: required the presence of a corneal ring infiltrate and 1 or more features of stage 2 disease.
Source: Table 14.2.1.6

Table 9. Demographics (SAS)

	0.8 mg/ml polihexanide + placebo (N=69)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N=65)	Total (N=134)
Age (years)			
n/nmiss	69/0	65/0	134/0
Mean (SD)	35.1 (13.0)	37.9 (14.1)	36.5 (13.6)
Median	33.0	36.0	35.0
Q1, Q3	25.0, 43.0	26.0, 49.0	25.0, 46.0
Min, Max	15, 73	17, 71	15, 73
Sex			
Female	41 (59.4%)	37 (56.9%)	78 (58.2%)
Male	28 (40.6%)	28 (43.1%)	56 (41.8%)
Hypermetropes	8 (11.6%)	7 (10.8%)	15 (11.2%)
Emmetropes	2 (2.9%)	4 (6.2%)	6 (4.5%)
Myopes	55 (79.7%)	51 (78.5%)	106 (79.1%)

n/nmiss = number of subjects with evaluable/missing data, Q1 = first quartile, Q3 = third quartile, SD = standard deviation
Percentages are based on the number of subjects within each treatment group
Program: \Subprogs\Tables\DM.sas
Date and time program was run: 2022-03-09T08:27. Date and time analysis database was run: 2021-11-30T18:15

Prior Medication

Overall, a majority (93/134; 69.4%) of subjects had at least one prior medication; 52 subjects (75.4%) in 0.8 mg/ml polihexanide monotherapy and 41 subjects (63.1%) in the 0.2 mg/ml polihexanide + 1 mg/ml propamidine combination therapy group.

Table 10. List of prior medications (Safety analysis set)

Therapeutic subgroup/Preferred name	0.8 mg/ml polihexanide + placebo (N=69)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N=65)	Total (N=134)
Any prior medication	**52 (75.4%)**	**41 (63.1%)**	**93 (69.4%)**
OPHTHALMOLOGICALS	**52 (75.4%)**	**40 (61.5%)**	**92 (68.7%)**
Fluoroquinolones	25 (36.2%)	20 (30.8%)	45 (33.6%)
Antibiotics	19 (27.5%)	21 (32.3%)	40 (29.9%)
Antivirals	17 (24.6%)	16 (24.6%)	33 (24.6%)
Corticosteroids, plain	17 (24.6%)	11 (16.9%)	28 (20.9%)
Corticosteroids and antiinfectives in combination	16 (23.2%)	5 (7.7%)	21 (15.7%)
Anticholinergics	11 (15.9%)	4 (6.2%)	15 (11.2%)
Other ophthalmologicals	5 (7.2%)	5 (7.7%)	10 (7.5%)
Antiinflammatory agents, non-steroids	1 (1.4%)	1 (1.5%)	2 (1.5%)
Other antiinfectives	1 (1.4%)	1 (1.5%)	2 (1.5%)
Viscoelastic substances	2 (2.9%)	0	2 (1.5%)
ANTIVIRALS FOR SYSTEMIC USE	**6 (8.7%)**	**8 (12.3%)**	**14 (10.4%)**
Nucleosides and nucleotides excl. reverse transcriptase inhibitors	6 (8.7%)	8 (12.3%)	14 (10.4%)
ANTIBACTERIALS FOR SYSTEMIC USE	**3 (4.3%)**	**2 (3.1%)**	**5 (3.7%)**
Combinations of sulfonamides and trimethoprim, incl. Derivatives	2 (2.9%)	1 (1.5%)	3 (2.2%)
Carbapenems	1 (1.4%)	0	1 (0.7%)
Fluoroquinolones	0	1 (1.5%)	1 (0.7%)
Glycopeptide antibacterials	1 (1.4%)	0	1 (0.7%)
Third-generation cephalosporins	0	1 (1.5%)	1 (0.7%)
ANTISEPTICS AND DISINFECTANTS	**3 (4.3%)**	**1 (1.5%)**	**4 (3.0%)**
Iodine products	3 (4.3%)	1 (1.5%)	4 (3.0%)
ANTIINFLAMMATORY AND ANTIRHEUMATIC PRODUCTS	**1 (1.4%)**	**1 (1.5%)**	**2 (1.5%)**
Acetic acid derivatives and related substances	1 (1.4%)	0	1 (0.7%)

Therapeutic subgroup/Preferred name	0.8 mg/ml polihexanide + placebo (N=69)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N=65)	Total (N=134)
Other antiinflammatory and antirheumatic agents, non-steroids	0	1 (1.5%)	1 (0.7%)
CORTICOSTEROIDS FOR SYSTEMIC USE	**1 (1.4%)**	**1 (1.5%)**	**2 (1.5%)**
Corticosteroids for systemic use, combinations	0	1 (1.5%)	1 (0.7%)
Glucocorticoids	1 (1.4%)	0	1 (0.7%)
ANALGESICS	**1 (1.4%)**	**0**	**1 (0.7%)**
Anilides	1 (1.4%)	0	1 (0.7%)
ANTIHISTAMINES FOR SYSTEMIC USE	**1 (1.4%)**	**0**	**1 (0.7%)**
Other antihistamines for systemic use	1 (1.4%)	0	1 (0.7%)
ANTIMYCOTICS FOR SYSTEMIC USE	**1 (1.4%)**	**0**	**1 (0.7%)**
Triazole derivatives	1 (1.4%)	0	1 (0.7%)
Ophthalmological and otological preparations	**0**	**1 (1.5%)**	**1 (0.7%)**
Antiinfectives	0	1 (1.5%)	1 (0.7%)
PREPARATIONS FOR TREATMENT OF WOUNDS AND ULCERS	**0**	**1 (1.5%)**	**1 (0.7%)**
Other cicatrizants	0	1 (1.5%)	1 (0.7%)
antimycobacterials	**0**	**1 (1.5%)**	**1 (0.7%)**
Antibiotics	0	1 (1.5%)	1 (0.7%)
ophthalmologicals	**0**	**1 (1.5%)**	**1 (0.7%)**
Antibiotics	0	1 (1.5%)	1 (0.7%)
Carbonic anhydrase inhibitors	0	1 (1.5%)	1 (0.7%)
Other ophthalmologicals	0	1 (1.5%)	1 (0.7%)
Sympathomimetics used as decongestants	0	1 (1.5%)	1 (0.7%)

Medications are coded according to <WHODD/ATC Index> <version>.
Percentages are based on the number of subjects within each treatment group
Program: \Subprogs\Tables\CM.sas
Date and time program was run: 2022-03-09T08:27. Date and time analysis database was run: 2021-11-30T18:15

Use of additional medications

The use of additional medications during the study was required in 112/134 (83.6%) of subjects. Mainly were ophthalmic drugs. Ophthalmic fluoroquinolones were used in 70/134 (52.2%) of subjects, plain corticosteroids in 45/134 (33.6%), other ophthalmologicals in 42/134 (31.3%), anticholinergics in 22/134 (16.4%), antiseptics and disinfectants in 22/134 (16.4%) and other antibiotics in 20/134 (14.9%). Most used systemic medications were non-steroidal anti-inflammatory drugs used in 53/134 (39.6%) of subjects, and analgesics 35/134 (26.1%).

Table 11. List of concomitant medication (Study 043, Safety analysis set)

Therapeutic subgroup/Preferred name	0.8 mg/mL polihexanide + placebo (N=69)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=65)	Total (N=134)
Any concomitant medication	**54 (78.3%)**	**58 (89.2%)**	**112 (83.6%)**
OPHTHALMOLOGICALS	**50 (72.5%)**	**50 (76.9%)**	**100 (74.6%)**
Fluoroquinolones	35 (50.7%)	35 (53.8%)	70 (52.2%)
Corticosteroids, plain	25 (36.2%)	20 (30.8%)	45 (33.6%)
Other ophthalmologicals	20 (29.0%)	22 (33.8%)	42 (31.3%)
Anticholinergics	10 (14.5%)	12 (18.5%)	22 (16.4%)
Antibiotics	11 (15.9%)	9 (13.8%)	20 (14.9%)
Other antiinfectives	12 (17.4%)	5 (7.7%)	17 (12.7%)
Viscoelastic substances	3 (4.3%)	3 (4.6%)	6 (4.5%)
Antivirals	0	4 (6.2%)	4 (3.0%)
Beta blocking agents	1 (1.4%)	2 (3.1%)	3 (2.2%)
Corticosteroids and antiinfectives in combination	1 (1.4%)	2 (3.1%)	3 (2.2%)
Other antiallergics	0	2 (3.1%)	2 (1.5%)
Carbonic anhydrase inhibitors	1 (1.4%)	0	1 (0.7%)
ANTIINFLAMMATORY AND ANTIRHEUMATIC PRODUCTS	**29 (42.0%)**	**24 (36.9%)**	**53 (39.6%)**
Acetic acid derivatives and related substances	22 (31.9%)	15 (23.1%)	37 (27.6%)
Propionic acid derivatives	11 (15.9%)	14 (21.5%)	25 (18.7%)
ANALGESICS	**16 (23.2%)**	**19 (29.2%)**	**35 (26.1%)**
Anilides	11 (15.9%)	11 (16.9%)	22 (16.4%)
Opioids in combination with non-opioid analgesics	6 (8.7%)	11 (16.9%)	17 (12.7%)
Natural opium alkaloids	1 (1.4%)	1 (1.5%)	2 (1.5%)
Other analgetics and antipyretics	1 (1.4%)	0	1 (0.7%)
ANTISEPTICS AND DISINFECTANTS	**13 (18.8%)**	**9 (13.8%)**	**22 (16.4%)**
Biguanides and amidines	13 (18.8%)	9 (13.8%)	22 (16.4%)
ANTIVIRALS FOR SYSTEMIC USE	**9 (13.0%)**	**3 (4.6%)**	**12 (9.0%)**
Nucleosides and nucleotides excl. reverse transcriptase inhibitors	9 (13.0%)	3 (4.6%)	12 (9.0%)
SEX HORMONES AND MODULATORS OF THE GENITAL SYSTEM	**3 (4.3%)**	**6 (9.2%)**	**9 (6.7%)**
Progestogens	3 (4.3%)	4 (6.2%)	7 (5.2%)
Antiandrogens and estrogens	0	1 (1.5%)	1 (0.7%)
Progestogens and estrogens, sequential preparations	0	1 (1.5%)	1 (0.7%)
PSYCHOANALEPTICS	**5 (7.2%)**	**3 (4.6%)**	**8 (6.0%)**
Selective serotonin reuptake inhibitors	4 (5.8%)	3 (4.6%)	7 (5.2%)
Other antidepressants	1 (1.4%)	0	1 (0.7%)
ANTIBACTERIALS FOR SYSTEMIC USE	**4 (5.8%)**	**2 (3.1%)**	**6 (4.5%)**
Tetracyclines	2 (2.9%)	1 (1.5%)	3 (2.2%)
Fluoroquinolones	1 (1.4%)	0	1 (0.7%)

Therapeutic subgroup/Preferred name	0.8 mg/mL polihexanide + placebo (N=69)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=65)	Total (N=134)
Second generation cephalosporins	1 (1.4%)	0	1 (0.7%)
Trimethoprim and derivatives	0	1 (1.5%)	1 (0.7%)
DRUGS FOR ACID RELATED DISORDERS	**3 (4.3%)**	**3 (4.6%)**	**6 (4.5%)**
Proton pump inhibitors	3 (4.3%)	3 (4.6%)	6 (4.5%)
ANTIHISTAMINES FOR SYSTEMIC USE	**5 (7.2%)**	**0**	**5 (3.7%)**
Other antihistamines for systemic use	3 (4.3%)	0	3 (2.2%)
Piperazine derivatives	2 (2.9%)	0	2 (1.5%)
PSYCHOLEPTICS	**0**	**3 (4.6%)**	**3 (2.2%)**
Benzodiazepine related drugs	0	2 (3.1%)	2 (1.5%)
Benzodiazepine derivatives	0	1 (1.5%)	1 (0.7%)
THYROID THERAPY	**2 (2.9%)**	**1 (1.5%)**	**3 (2.2%)**
Thyroid hormones	2 (2.9%)	1 (1.5%)	3 (2.2%)
VITAMINS	**3 (4.3%)**	**0**	**3 (2.2%)**
Ascorbic acid (vitamin C), plain	3 (4.3%)	0	3 (2.2%)
OPHTHALMOLOGICALS	**3 (4.3%)**	**0**	**3 (2.2%)**
Local anesthetics	2 (2.9%)	0	2 (1.5%)
Other ophthalmologicals	1 (1.4%)	0	1 (0.7%)
ANTITHROMBOTIC AGENTS	**1 (1.4%)**	**1 (1.5%)**	**2 (1.5%)**
Platelet aggregation inhibitors excl. Heparin	0	1 (1.5%)	1 (0.7%)
Vitamin K antagonists	1 (1.4%)	0	1 (0.7%)
BETA BLOCKING AGENTS	**1 (1.4%)**	**1 (1.5%)**	**2 (1.5%)**
Beta blocking agents, non-selective	1 (1.4%)	0	1 (0.7%)
Beta blocking agents, selective	0	1 (1.5%)	1 (0.7%)
AGENTS ACTING ON THE RENIN-ANGIOTENSIN SYSTEM	**0**	**1 (1.5%)**	**1 (0.7%)**
ACE inhibitors, plain	0	1 (1.5%)	1 (0.7%)
ANTI-PARKINSON DRUGS	**0**	**1 (1.5%)**	**1 (0.7%)**
Dopa and dopa derivatives	0	1 (1.5%)	1 (0.7%)
ANTIMYCOTICS FOR SYSTEMIC USE	**0**	**1 (1.5%)**	**1 (0.7%)**
Triazole derivatives	0	1 (1.5%)	1 (0.7%)
DRUGS USED IN DIABETES	**0**	**1 (1.5%)**	**1 (0.7%)**
Insulins and analogues for injection, fast-acting	0	1 (1.5%)	1 (0.7%)
Insulins and analogues for injection, long-acting	0	1 (1.5%)	1 (0.7%)
LIPID MODIFYING AGENTS	**0**	**1 (1.5%)**	**1 (0.7%)**
HMG Coa reductase inhibitors	0	1 (1.5%)	1 (0.7%)
OPHTHALMOLOGICAL AND OTOLOGICAL PREPARATIONS	**1 (1.4%)**	**0**	**1 (0.7%)**
Antiinfectives	1 (1.4%)	0	1 (0.7%)
ANTI ACNE PREPARATIONS	**0**	**1 (1.5%)**	**1 (0.7%)**
Retinoids for treatment of acne	0	1 (1.5%)	1 (0.7%)

Therapeutic subgroup/Preferred name	0.8 mg/mL polihexanide + placebo (N=69)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=65)	Total (N=134)
DRUGS FOR OBSTRUCTIVE AIRWAY DISEASE	**0**	**1 (1.5%)**	**1 (0.7%)**
Glucocorticoids	0	1 (1.5%)	1 (0.7%)
Selective beta-2-adrenoreceptor agonists	0	1 (1.5%)	1 (0.7%)
MINERAL SUPPLEMENTS	**1 (1.4%)**	**0**	**1 (0.7%)**
Potassium	1 (1.4%)	0	1 (0.7%)
PHSYCHOANALEPTICS	**1 (1.4%)**	**0**	**1 (0.7%)**
Centrally acting sympathomimetics	1 (1.4%)	0	1 (0.7%)
THROAT PREPARATIONS	**0**	**1 (1.5%)**	**1 (0.7%)**
Antibiotics	0	1 (1.5%)	1 (0.7%)
UROLOGICALS	**0**	**1 (1.5%)**	**1 (0.7%)**
Alpha-adrenoreceptor antagonists	0	1 (1.5%)	1 (0.7%)
VACCINES	**0**	**1 (1.5%)**	**1 (0.7%)**
Other viral vaccines	0	1 (1.5%)	1 (0.7%)

Source: Study 043/SI CSR, Table 14.1.4.2.
Medications were coded according to the World Health Organization (WHO) anatomical therapeutic chemical (ATC) classification system and summarized by therapeutic subgroup (ATC level 2) and preferred name

Table 12. Rate of subjects requiring adjunctive therapy

	0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=61)
Rate of subjects with Adjunctive therapy (%)	30 (45.5%)	25 (41.0%)
Rate of subjects with Adjunctive therapy (%) and having steroids after randomization	11 (16.7%)	13 (21.3%)

Source: Study 043/SI CSR, Table 14.3.2.1.
Note: Adjunctive therapy is defined as new use after baseline or any increase of at least one of following medication with ATC5:
Topical steroid: dexamethasone, prednisolone, fluorometholone, loteprednol
Oral non-steroidal anti-inflammatories (NSAIDs): diclofenac, ibuprofen, flurbiprofen, naproxen

Treatment compliance

Table 13. Study Drug Administration Compliance with the Protocol (FAS)

	0.8 mg/ml polihexanide + placebo (N = 66)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N = 61)	Total (N = 127)
No	4 (6.1%)	3 (4.9%)	7 (5.5%)
Yes	62 (93.9%)	58 (95.1%)	120 (94.5%)
Percentages are based on the number of subjects within each treatment group. Source: Table 14.1.5.1			

- **Numbers analysed**

The FAS consisted of 127 subjects, the PPAS consisted of 119 subjects, and the SS consisted of 134 subjects.

Table 14. Analysis Populations

	0.8 mg/ml polihexanide + placebo	0.2 mg/ml polihexanide + 1 mg/ml propamidine	All subjects
Safety Analysis Set	69	65	134
Full Analysis Set	66	61	127
Per Protocol Analysis Set	62	57	119
Source: Table 14.1.1.1			

Table 15. Reason for premature withdrawals from study: Full analysis set

	0.8 mg/ml polihexanide + placebo (N=66)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N=61)	Total (N=127)
Prematurely withdrawn from the study[a]	10 (15.2%)	7 (11.5%)	17 (13.4%)
Primary reason for withdrawal[b]			
Adverse event	7 (70.0%)	7 (100.0%)	14 (82.4%)
Lost to follow-up	1 (10.0%)	0	1 (5.9%)
Ocular intolerance	1 (10.0%)	0	1 (5.9%)
Other	1 (10.0%)	0	1 (5.9%)

[a]Percentages are based on the number of subjects within each treatment group
[b]Percentages are based on the number of prematurely withdrawn patients within each treatment group.
Patient 11-49 has Other reason for not completing the study and is specified as: Treatment Failure After 12 Months Of Study
Program: \Subprogs\Tables\DS Disposition.sas
Date and time program was run: 2022-03-09T08:26. Date and time analysis database was run: 2021-11-30T18:15

Table 16. Reason for discontinuation from study treatment: Full analysis set

	0.8 mg/ml polihexanide + placebo (N=66)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N=61)	Total (N=127)
Discontinuation from the study treatment[a]	9 (13.6%)	7 (11.5%)	16 (12.6%)
Primary reason for discontinuation from the study treatment[b]			
Adverse event	7 (77.8%)	7 (100.0%)	14 (87.5%)
Other	1 (11.1%)	0	1 (6.3%)
Physician decision	1 (11.1%)	0	1 (6.3%)
[a]Percentages are based on the number of subjects within each treatment group [b]Percentages are based on the number of patients discontinuated from the study treatment within each treatment group. Other reason for discontinuation are specified in listing 16.2.1.1. Program: \Subprogs\Tables\DS Disposition.sas Date and time program was run: 2022-03-09T08:26. Date and time analysis database was run: 2021-11-30T18:15			

- **Outcomes and estimation**

Primary Efficacy Endpoint: 12-month clinical resolution rate

Table 17. Clinical resolution rate with 95%CIs (FAS)

	0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL propamidine (N=61)	Total (N=127)
Clinical resolution rate n (%)	**56 (84.8)**	**54 (88.5)**	**110 (86.6)**
Ratio (95% CI)	5.60 (2.86,10.98)	7.71 (3.51, 16.95)	
Odd ratio (95% CI)	0.73 (0.26, 2.04)		
p-value	0.544		
Difference in proportion of CRR (95% CI)	-0.04 (-0.16, 0.09)		
CI = Confidence interval			

Table 18. Clinical resolution rate with 95%CIs (PPAS)

	0.8 mg/mL polihexanide + placebo (N=62)	0.2 mg/mL polihexanide + 1.0 mg/mL propamidine (N=57)	Total (N=119)
Clinical resolution rate, n (%)	**54 (87.1)**	**51 (89.5)**	**105 (88.2)**
Ratio (95%CI)	6.75 (3.21,14.18)	8.50 (3.65, 19.81)	
Odd ratio (95% CI)	0.79 (0.26, 2.45)		
p-value	0.688		
Difference in proportion of CRR (95% CI)	-0.02 (-0.15, 0.10)		
CI = Confidence interval			

Table 19. Statistical analysis of 12-month clinical resolution rate with 90%CIs (FAS)

12-month clinical resolution rate (%)	0.8 mg/ml polihexanide + placebo (N = 66)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N = 61)	Total (N = 127)
Number of subjects included in analysis	66	61	
Resolved	56 (84.8%)	54 (88.5%)	110 (86.6%)
Not resolved	10 (15.2%)	7 (11.5%)	17 (13.4%)
Ratio (90% CI)	5.60 (3.18, 9.85)	7.71 (3.98, 14.94)	
Odds Ratio (90% CI)	0.73 (0.30, 1.73)		
p-value	0.544		
Test for non-inferiority: Differences in proportion of clinical resolution rate (90% CI)	-0.04 (-0.14, 0.07)		

CI = confidence interval
Result displays ratio comparison of (0. 8 mg/ml PHMB + placebo)/(0.2 mg/ml PHMB + 1 mg/ml propamidine) and is based on logistic regression model.
The model is modelling the probability that the subject has reached a clinical resolution (CR). M eaning that if OR > 1 the 0.8 mg/ml PHMB + placebo is estimated to, on average, have a higher probability of reaching CR in comparison to 0.2 mg/ml PHMB + 1 mg/ml propamidine.
Null hypothesis non-inferiority: Test treatment is inferior to the reference by delta = 0.20 or more. That means if the lower limit of the 90% CI is above -
0.2 the null hypothesis is rejected and non-inferiority is met
Source: Table 14.2.1.1

Table 20. Statistical analysis of 12-month clinical resolution rate with 90% CIs (PPAS)

12-month clinical resolution rate (%)	0.8 mg/ml polihexanide + placebo (N = 62)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N = 57)	Total (N = 119)
Resolved	54 (87.1%)	51 (89.5%)	105 (88.2%)
Not resolved	8 (12.9%)	6 (10.5%)	14 (11.8%)
Ratio (90% CI)	6.75 (3.62, 12.59)	8.50 (4.18, 17.29)	
Odds Ratio (90% CI)	0.79 (0.31, 2.04)		
p-value	0.688		
Test for non-inferiority: Differences in proportion of clinical resolution rate (90% CI)	-0.02 (-0.13, 0.08)		

CI = confidence interval
Result displays ratio comparison of (0. 8 mg/ml PHMB + placebo)/(0.2 mg/ml PHMB + 1 mg/ml propamidine) and is based on logistic regression model.
The model is modelling the probability that the subject has reached a clinical resolution (CR). Meaning that if OR > 1 the 0.8 mg/ml PHMB + placebo is estimated to, on average, have a higher probability of reaching CR in comparison to 0.2 mg/ml PHMB + 1 mg/ml propamidine.
Null hypothesis non-inferiority: Test treatment is inferior to the reference by delta = 0.20 or more. That means if the lower limit of the 90% CI is above -0.2 the null hypothesis is rejected and non-inferiority is met
Source: Table 14.2.1.2

Secondary efficacy endpoints

Time to cure

Figure 4. Kaplan-Meier curve of Time-to-cure (FAS)

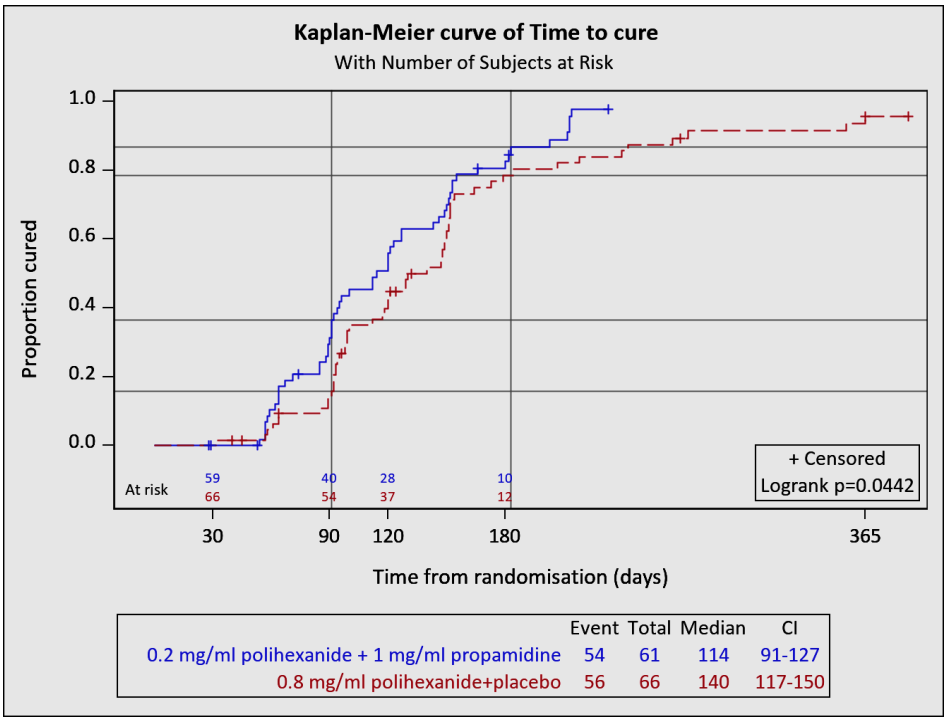


Table 21. Time-to-cure, patients with clinical resolution (FAS)

Time-to-a-cure (days)	0.8 mg/ml polihexanide + placebo (N=56)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N=54)
End-of-study visit		
n/nmiss	56/0	54/0
Mean (SD)	138.3 (67.3)	117.1 (47.0)
Median	125.0	112.0
Q1, Q3	93.0, 152.0	85.0, 151.0
Min, Max	32, 365	54, 214
p-value for difference between treatment groups[1]	0.0934	

n/nmiss = number of subjects with evaluable/missing data, Q1 = first quartile, Q3 = third quartile, SD = standard deviation
Table includes patients who have been cured.
[1]Mann Whitney U test.
Program: \Subprogs\Tables\EFF2 time-to-cure v2.sas
Date and time program was run: 2022-03-09T08:28. Date and time analysis database was run: 2021-11-30T18:15

Table 22. Time-to-cure, all patients (FAS)

Time-to-a-cure (days)	0.8 mg/ml polihexanide + placebo (N=66)	0.2 mg/ml polihexanide + 1 mg/ml Propamidine (N=61)
Number of subjects cured	56	54
Number of subjects censored	10	7
Reason for censoring		
Physician decision	1	0
Adverse event	3	3
Lost to follow-up	1	0
Treatment failure	5	4
Median time to cure (95% CI)	140 (117, 150)	114 (91, 127)
Log-rank test p-value	0.0442	

CI = confidence interval
Median time to cure is the Kaplan-Meier estimated median survival time.
Log-rank test of difference in survival function between treatments

Table 23. Time-to-cure (FAS), patients with clinical resolution

12-month clinical resolution rate		0.8 mg/ml polihexanide + placebo (N=66)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N=61)	Total (N=127)
Resolved	Within 3 months	13 (23.2%)	22 (40.7%)	35 (31.8%)
Resolved	Between 3 and 6 months	34 (60.7%)	27 (50.0%)	61 (55.5%)
Resolved	More than 6 months	9 (16.1%)	5 (9.3%)	14 (12.7%)
p-value	Chi-Square test for difference across treatment			0.3764

Percentages are based on the number of subjects with clinical resolution and treatment
Program: \Subprogs\Tables\crr_5T.sas
Date and time program was run: 2022-03-09T08:28. Date and time analysis database was run: 2021-11-30T18:15

Table 24. Clinical resolution rate at intermediate time points (FAS)

		0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL propamidine (N=61)
Clinical resolution rate at 6 months			
n		47	49
% CRR (95% CI)		71.2 % (58.7, 81.7)	80.3 % (68.2, 89.4)
Clinical resolution rate at 5 months			
n		43	44
% CRR (95% CI)		65.2 % (52.4, 76.5)	72.1 % (59.2, 82.9)
Clinical resolution rate at 4 months			
n		28	33
% CRR (95% CI)		42.4% (30.3, 55.2)	54.1% (40.8, 66.9)
Clinical resolution rate at 3 months			
n		13	22
% CRR (95% CI)		19.7% (10.9, 31.3)	36.1% (24.2, 49.4)
Clinical resolution rate at 2 months			
n		4	6
% CRR (95% CI)		6.1% (1.7, 14.8)	9.8% (3.7, 20.2)
Clinical resolution rate at 1 month			
n		0	0
% CRR (95% CI)		0	0
CI= Confidence interval; CRR= Clinical resolution rate			

Table 25. Global length of exposure (Study 043, SAS)

Duration (days)	0.08% PHMB + placebo (N=69)	0.02% PHMB + 0.1% Propamidine (N=65)	Total (N=134)
Total (all visits)			
n/nmiss	69/0	65/0	134/0
Mean (SD)	138.3 (79.6)	113.9 (52.5)	126.4 (68.7)
Median	120.0	100.0	115.5
Q1, Q3	92.0, 152.0	71.0, 152.0	89.0, 152.0
Min, Max	10, 387	28, 233	10, 387

Source: Study 043/SI CSR, Table 14.3.1.1.
If a patient had a missing end of treatment date, the end of study date was used to calculate duration.
n/nmiss = number of subjects with evaluable/missing data; Q1 = first quartile; Q3 = third quartile;
SD = standard deviation.

BCVA

Table 26. Statistical analysis of BCVA through the Log MAR score (FAS)

BCVA Log MAR score	0.8 mg/ml polihexanide + placebo (N=64)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N=60)
End-of-study visit		
Number of subjects included in analysis	53	46
Mean change from Baseline in Log MAR score (90% CI)	-0.25 (-0.32, -0.18)	-0.30 (-0.38, -0.22)
Difference (90% CI)	0.05 (-0.06, 0.16)	
p-value	0.444	

CI = confidence interval
Results display comparison between respective groups and are based on the ANCOVA model.
Patient 31-04 31-08 and 31-10 was removed form the analyses due to wrong values in the eCRF.
Program: \Subprogs\Tables\BCVA.sas
Date and time program was run: 2022-03-09T08:28. Date and time analysis database was run: 2021-11-30T18:15

Corneal scarring

Table 27. Corneal scarring (Study 043, ITT analysis set)

	0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=61)	Total (N=127)
Baseline			
Absent	66 (100.0%)	58 (95.1%)	124 (97.6%)
Present	0	3 (4.9%)	3 (2.4%)
Missing	0	0	0
End-of-study visit			
Absent	31 (47.0%)	25 (41.0%)	56 (44.1%)
Present	33 (50.0%)	30 (49.2%)	63 (49.6%)
Missing	2 (3.0%)	6 (9.8%)	8 (6.3%)
McNemar's test p-value for difference from baseline within treatment group	<0.0001	<0.0001	<0.0001
Shift from baseline to End-of-study visit			
Absent to Absent	31 (47.0%)	24 (39.3%)	55 (43.3%)
Absent to Present	33 (50.0%)	29 (47.5%)	62 (48.8%)
Present to Absent	0	1 (1.6%)	1 (0.8%)
Present to Present	0	1 (1.6%)	1 (0.8%)
Missing at any of these visits	2 (3.0%)	6 (9.8%)	8 (6.3%)
Odds Ratio (95% CI) [1]	1.14 (0.55, 2.36)		
p-value	0.734		

Source: Study 043/SI CSR, Tables 14.2.4.9 and 14.2.4.10.
CI = confidence interval.
[1] Ratio comparison is based on logistic regression model using treatment group and baseline as predictors. Number of subjects included in the analysis are those with a baseline and an end of the study value (n=64 and n= 55, in 0.8 mg/mL polihexanide + placebo and 0.2 mg/mL polihexanide + 1.0 mg/mL propamidine, respectively). The model is modelling the probability that the parameter is absent.

Corneal ulceration

Table 28. Corneal ulceration (Study 043, ITT analysis set)

	0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=61)	Total (N=127)
Baseline			
Absent	48 (72.7%)	45 (73.8%)	93 (73.2%)
Present	18 (27.3%)	16 (26.2%)	34 (26.8%)
Missing	0	0	0
End-of-study visit			
Absent	60 (90.9%)	52 (85.2%)	112 (88.2%)
Present	4 (6.1%)	3 (4.9%)	7 (5.5%)
Missing	2 (3.0%)	6 (9.8%)	8 (6.3%)
McNemar's test p-value for difference from baseline within treatment group	0.001	0.0047	<0.0001
Shift from baseline to End-of-study visit			
Absent to Absent	44 (66.7%)	44 (72.1%)	88 (69.3%)
Absent to Present	2 (3.0%)	0	2 (1.6%)
Present to Absent	16 (24.2%)	8 (13.1%)	24 (18.9%)
Present to Present	2 (3.0%)	3 (4.9%)	5 (3.9%)
Missing at any of these visits	2 (3.0%)	6 (9.8%)	8 (6.3%)
Odds Ratio (95% CI) [1]	1.09 (0.22, 5.47)		
p-value	0.917		

Source: Study 043/SI CSR, Tables 14.2.4.3 and 14.2.4.4.
CI = confidence interval.
[1] Ratio comparison is based on logistic regression model using treatment group and baseline as predictors. Number of subjects included in the analysis are those with a baseline and an end of the study value (n=64 and n= 55, in 0.8 mg/mL polihexanide + placebo and 0.2 mg/mL polihexanide + 1.0 mg/mL propamidine, respectively).The model is modelling the probability that the parameter is absent.

Pupil test

Table 29. Pupil test (FAS)

Pupil test	0.8 mg/ml polihexanide + placebo (N=66)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N=61)	Total (N=127)
Baseline			
Normal	66 (100.0%)	60 (98.4%)	126 (99.2%)
Abnormal, NCS	0	0	0
Abnormal, CS	0	0	0
Missing	0	1 (1.6%)	1 (0.8%)
End-of-study visit			
Normal	62 (93.9%)	54 (88.5%)	116 (91.3%)
Abnormal, NCS	1 (1.5%)	1 (1.6%)	2 (1.6%)
Abnormal, CS	0	0	0
Missing	3 (4.5%)	6 (9.8%)	9 (7.1%)

CS = clinically significant, NCS = not clinically significant
Baseline is defined as the last valid evaluation prior to the first dose of study medication.
Percentages are based on the number of subjects within each treatment group
Program: \Subprogs\Tables\Pupil test.sas
Date and time program was run: 2022-03-09T08:28. Date and time analysis database was run: 2021-11-30T18:15

Anterior chamber flare assessment

Table 30. Anterior chamber flare (Study 043, ITT analysis set)

	0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=61)	Total (N=127)
Baseline			
None	52 (78.8%)	44 (72.1%)	96 (75.6%)
Mild	11 (16.7%)	11 (18.0%)	22 (17.3%)
Moderate	1 (1.5%)	4 (6.6%)	5 (3.9%)
Severe	1 (1.5%)	1 (1.6%)	2 (1.6%)
Missing	1 (1.5%)	1 (1.6%)	2 (1.6%)
End-of-study visit			
None	62 (93.9%)	50 (82.0%)	112 (88.2%)
Mild	1 (1.5%)	1 (1.6%)	2 (1.6%)
Moderate	0	1 (1.6%)	1 (0.8%)
Severe	1 (1.5%)	1 (1.6%)	2 (1.6%)
Missing	2 (3.0%)	8 (13.1%)	10 (7.9%)
Paired T-test p-value for change from baseline within treatment group	0.0221	0.0100	0.0005
Shift from baseline to End-of-study visit			
None to None	49 (74.2%)	36 (59.0%)	85 (66.9%)
None to Mild	1 (1.5%)	0	1 (0.8%)
None to Moderate	0	0	0
None to Severe	0	1 (1.6%)	1 (0.8%)
Mild to None	10 (15.2%)	10 (16.4%)	20 (15.7%)
Mild to Mild	0	1 (1.6%)	1 (0.8%)
Mild to Moderate	0	0	0
Mild to Severe	1 (1.5%)	0	1 (0.8%)
Moderate to None	1 (1.5%)	3 (4.9%)	4 (3.1%)
Moderate to Mild	0	0	0
Moderate to Moderate	0	1 (1.6%)	1 (0.8%)
Severe to None	1 (1.5%)	1 (1.6%)	2 (1.6%)
Severe to Mild	0	0	0
Severe to Moderate	0	0	0
Missing at any of these visits	3 (4.5%)	8 (13.1%)	11 (8.7%)
Odds Ratio (95% CI) [1]	0.65 (0.14, 3.11)		
p-value	0.651		

Source: Study 043/SI CSR, Tables 14.2.4.4.1 and 14.2.4.4.2.
CI = confidence interval.
[1] Ratio comparison is based on ordinal logistic regression model using treatment group and baseline as predictors. Number of subjects included in the analysis are those with a baseline and an end of the study value (n=64 and n= 53, in 0.8 mg/mL polihexanide + placebo and 0.2 mg/mL polihexanide + 1.0 mg/mL propamidine, respectively).
The model is modelling the probability that the parameter is descending.

EQ-5D

Table 31. EQ-5D questionaire: VAS score (Study 43, ITT analysis set)

	0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=61)
Baseline		
N	64	58
Mean (SD)	69.8 (19.5)	70.7 (23.2)
Median	73.0	77.5
Q1, Q3	57.5, 85.0	60.0, 90.0
Min, Max	10, 100	10, 100
End-of-study visit		
N	61	55
Mean (SD)	88.3 (13.4)	90.9 (10.6)
Median	95.0	95.0
Q1, Q3	80.0, 99.0	80.0, 100.0
Min, Max	40, 100	50, 100
Change from baseline to End-of-study visit		
N	60	52
Mean (SD)	17.9 (19.6)	18.8 (20.0)
Median	14.5	15.0
Q1, Q3	5.0, 28.0	5.0, 30.0
Min, Max	-20, 65	-8, 90
p-value [1]	<0.0001	<0.0001
LS Mean (90% CI)	88.28 (85.84, 90.73)	90.25 (87.62, 92.88)
Difference (90% CI)	-1.97 (-5.56, 1.63)	
p-value [2]	0.366	

Source: Study 043/SI CSR, Tables 14.2.5.1 and 14.2.5.2.

Percentages are based on the number of subjects within each treatment group.

CI = confidence interval, Q1 = first quartile, Q3 = third quartile,LS = least squares

[1] The p-values are based on paired t-tests where the baseline value is compared to the value at End-of-study.

[2] Results display comparison between respective groups and are based on the ANCOVA model. The model included the end-of-study EQ-5D value as dependent variable, treatment group as fixed effect and EQ-5D value at Baseline as covariate.

VFQ-25

Table 32. VFQ-25 questionnaire: Composite score (Study 043, ITT analysis set)

VFQ-25 composite score	0.8 mg/mL polihexanide + placebo (N=65)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=61)
Baseline		
N	64	61
Mean (SD)	64.9 (22.3)	64.8 (22.1)
Median	63.3	68.9
Q1, Q3	48.7, 84.8	48.3, 83.4
Min, Max	15, 97	12, 98
End-of-study visit		
N	60	55
Mean (SD)	88.1 (15.6)	89.9 (16.6)
Median	93.7	95.9
Q1, Q3	83.7, 97.8	89.8, 99.4
Min, Max	22, 100	13, 100
Change from baseline to End-of-study visit		
N	60	55
Mean (SD)	23.5 (19.4)	23.7 (19.7)
Median	22.1	21.4
Q1, Q3	7.9, 37.3	7.7, 35.0
Min, Max	-10, 70	-9, 83
p-value [1]	<0.0001	<0.0001
p-value for change comparison [2]	0.6546	

Source: Study 043/SI CSR, Table 14.2.6.1.
The VFQ-25 composite score was recoded for statistical analysis being score 100 the best and score 0 the worst
N = number of subjects with evaluable data; Q1 = first quartile; Q3 = third quartile; SD = standard deviation.
Baseline is defined as the last valid evaluation prior to the first dose of study medication.
[1] The p-values are based on paired t-tests where the baseline value is compared to the value at End-of-study.
[2] Results display comparison between respective groups and are based on the ANCOVA model.

- **Ancillary analyses**

Table 33. Comparison of polihexanide 0.8 mg/ml with "untreated" patients ("putative placebo") with and without adding the maximal study effect

Treatment	Putative placebo	Akantior	
Source	Systematic literature review	Study 043/SI (0.8 mg/ml polihexanide + placebo group)	Mean difference in proportion in CRR (95% CI)
N	56	66	
Cured without surgery (N)	11	56	
CRR (95%CI)	19.6% (10.2%, 32.4%)	84.8% (73.9%, 92.5%)	**65.2% (49.3%, 77.5%)**
CRR including 30.7% study effect (95%CI)	50.3% (36.6%, 64.1%)	84.8% (73.9%, 92.5%)	**34.5% (16.8%, 49.8%)**

Abbreviations: CI, confidence interval; N, number of patients; CRR, clinical resolution rate
Notes: 95% CI is based on Clopper–Pearson interval 'exact' method.

Subgroup analyses

Table 34. 12-months clinical resolution rate. Staging of *Acanthamoeba* keratitis at baseline (FAS)

12-month clinical resolution rate		0.8 mg/ml polihexanide + placebo (N = 62)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N = 57)	Total (N = 119)
1: Stage-I	Resolved	12 (85.7%)	8 (100.0%)	20 (90.9%)
	Not resolved	2 (14.3%)	0	2 (9.1%)
Fisher exact test for difference between treatment	p-value			0.2797
2: Stage-II	Resolved	37 (90.2%)	40 (87.0%)	77 (88.5%)
	Not resolved	4 (9.8%)	6 (13.0%)	10 (11.5%)
Fisher exact test for difference between treatment	p-value			0.6353
3: Stage-III	Resolved	7 (63.6%)	6 (85.7%)	13 (72.2%)
	Not resolved	4 (36.4%)	1 (14.3%)	5 (27.8%)

12-month clinical resolution rate		0.8 mg/ml polihexanide + placebo (N = 62)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N = 57)	Total (N = 119)
Fisher exact test for difference between treatment	p-value			0.2545

Stage 1 AK: is defined as the presence of corneal epitheliopathy only.
Stage 2 AK: is defined by the presence of = 1 corneal epithelial defects, perineural infiltrate or stromal infiltrate in addition to stage 1 findings.
Stage 3 AK: require the presence of a corneal ring infiltrate and 1 or more features of stage 2 disease.
Percentages are based on the number of subjects within each subgroup and treatment
Source: Table 14.2.1.6

Table 35. 12-months clinical resolution rate. Patients with and without risk factors (FAS)

12-month clinical resolution rate		0.8 mg/ml polihexanide + placebo (N=66)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N=61)	Total (N=127)
Patient with a risk factor	Resolved	43 (81.1%)	44 (86.3%)	87 (83.7%)
	Not resolved	10 (18.9%)	7 (13.7%)	17 (16.3%)
Fisher exact test for difference between treatment	p-value			0.5983
Patient without a risk factor	Resolved	13 (100.0%)	10 (100.0%)	23 (100.0%)

*Any use of following medication before treatment is regarded as a risk factor:
Topical antifungals: amphotericin, voriconazole, chlorhexidine 0.2%, natamycin
Oral antifungals: voriconazole, itraconazole, fluconazole
Topical antivirals: aciclovir, gangciclovir
Oral antivirals: aciclovir, famciclovir, valaciclovir
Topical antibiotics: chloramphenicol, moxifloxacin, levofloxacin, ofloxacin, gatifloxacin, gentamicin, neomycin
Topical steroid potent: dexamathasone 0.1% or Prednisolone forte 1%)
Topical steroid mild: prednisolone 0.5%, FML (flurometholone), loteprednol.)

Percentages are based on the number of subjects within each subgroup and treatment
Program: \Subprogs\Tables\crr_2T.sas
Date and time program was run: 2022-03-09T08:27. Date and time analysis database was run: 2021-11-30T18:15

Table 36. 12-months clinical resolution rate. Patients with and without steroid treatment starting prior to IMP (FAS)

12-month clinical resolution rate		0.8 mg/ml polihexanide + placebo (N=66)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N=61)	Total (N=127)
Patient with prior steroid treatment	Not resolved	7 (22.6%)	2 (10.0%)	9 (17.6%)
	Resolved	24 (77.4%)	18 (90.0%)	42 (82.4%)
Fisher exact test for difference between treatment	p-value			0.4535
Patient without prior steroid treatment	Not resolved	3 (8.6%)	5 (12.2%)	8 (10.5%)
	Resolved	32 (91.4%)	36 (87.8%)	68 (89.5%)
Fisher exact test for difference between treatment	p-value			0.7188

*Any use of following medication starting before treatment:
Corticosteroids and antiinfectives in combination
Corticosteroids, plain

Percentages are based on the number of subjects within each subgroup and treatment
Program: \Subprogs\Tables\crr_3T.sas
Date and time program was run: 2022-03-09T08:27. Date and time analysis database was run: 2021-11-30T18:15

Table 37. 12-months clinical resolution rate. Patients without prior steroid treatment (FAS)

12-month clinical resolution rate		0.8 mg/ml polihexanide + placebo (N=66)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N=61)	Total (N=127)
Patient without prior steroid treatment and with steroid treatment starting after IMP	Not resolved	2 (18.2%)	3 (23.1%)	5 (20.8%)
	Resolved	9 (81.8%)	10 (76.9%)	19 (79.2%)
Fisher exact test for difference between treatment	p-value			1.0000
Patient without any steroid treatment	Not resolved	1 (4.2%)	2 (7.1%)	3 (5.8%)
	Resolved	23 (95.8%)	26 (92.9%)	49 (94.2%)
Fisher exact test for difference between treatment	p-value			1.0000

*Any use of following medication is defined as steroid treatment:
Corticosteroids and antiinfectives in combination
Corticosteroids, plain

Percentages are based on the number of subjects within each subgroup and treatment
Program: \Subprogs\Tables\crr_3_2T.sas
Date and time program was run: 2022-03-09T08:27. Date and time analysis database was run: 2021-11-30T18:15

Table 38. CRR and time-to-diagnosis (Study 043, ITT analysis set)

12-month clinical resolution rate		0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=61)	Total (N=127)
Delay (days) in diagnosis / treatment of acanthamoeba keratitis 0-1 days (n=48)	Resolved	20 (80.0%)	21 (91.3%)	41 (85.4%)
	Not resolved	5 (20.0%)	2 (8.7%)	7 (14.6%)
Fisher exact test for difference between treatment	p-value			0.1521
Delay (days) in diagnosis / treatment of acanthamoeba keratitis 2-11 days (n=34)	Resolved	14 (82.4%)	13 (76.5%)	27 (79.4%)
	Not resolved	3 (17.6%)	4 (23.5%)	7 (20.6%)
Fisher exact test for difference between treatment	p-value			0.7652
Delay (days) in diagnosis / treatment of acanthamoeba keratitis 12-149 days (n=45)	Resolved	22 (91.7%)	20 (95.2%)	42 (93.3%)
	Not resolved	2 (8.3%)	1 (4.8%)	3 (6.7%)
Fisher exact test for difference between treatment	p-value			0.6811

Source: Study 043/SI CSR, Table 14.2.1.7.
Percentages are based on the number of subjects within each subgroup and treatment.

Figure 5. Forest plot of odds ratios for subgroups defined by risk factors (FAS)

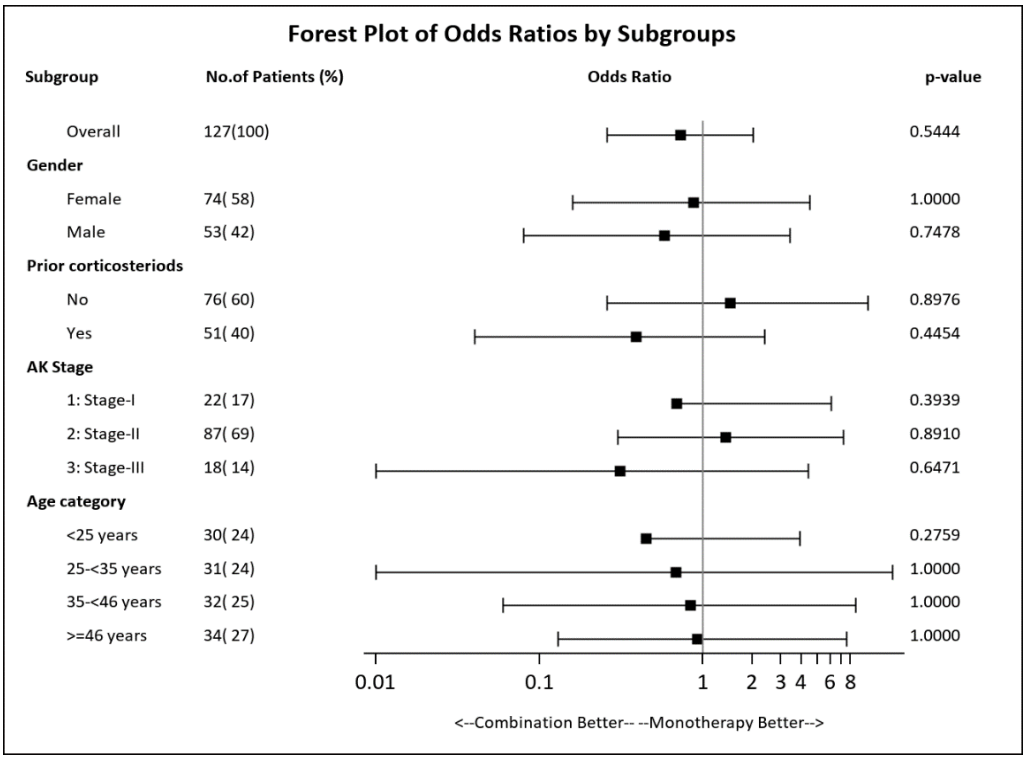
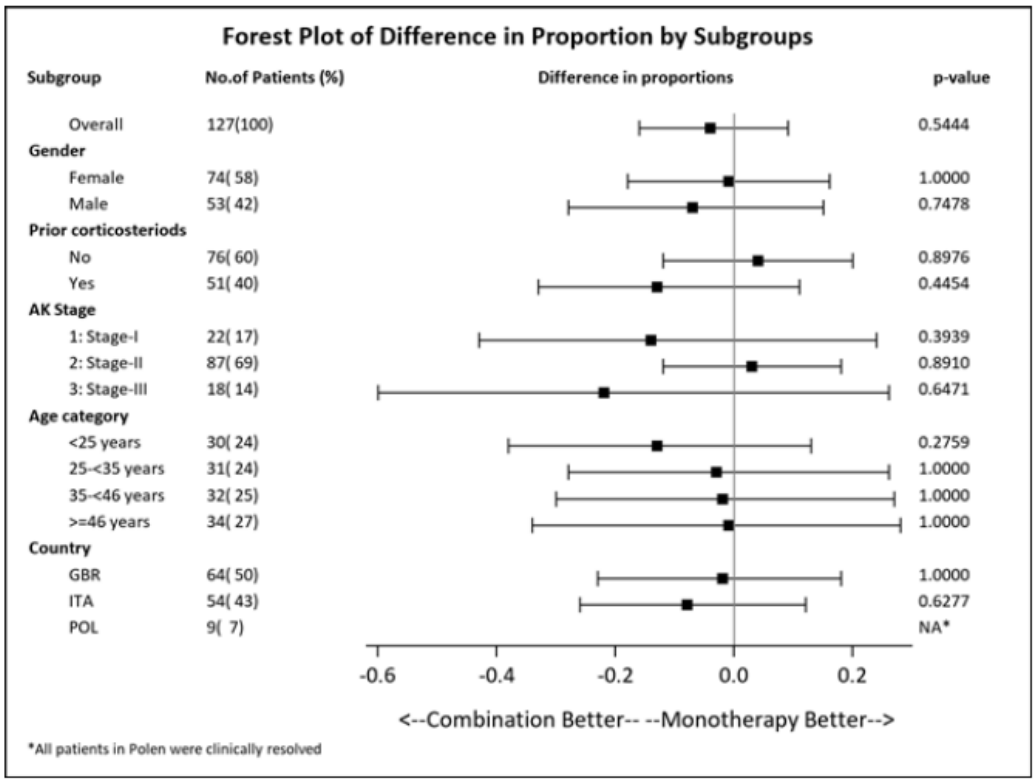


Figure 6. Forest plot of difference in proportion for CRR_12 for subgroups defined by risk factors (FAS)



AK = *Acanthamoeba* keratitis; CRR_12 = clinical resolution rate at 12 months from randomization; FAS = full analysis set; GBR = United Kingdom; ITA = Italy; POL/Polen = Poland.

Table 39. Clinical resolution rate for subgroups defined by risk factors (FAS)

		0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL propamidine (N=61)	
Risk factor		Cured (CRR_12)		Difference in proportion CRR_12 (95%CI)
Gender	Male (n=53)	22/27 (81.5%)	23/26 (88.5%)	-0.07 (-0.28, 0.15)
	Female (n=74)	34/39 (87.2%)	31/35 (88.6%)	-0.01 (-0.18, 0.16)
Prior use of steroids	Yes (n=51)	24/31 (77.4%)	18/20 (90.0%)	-0.13 (-0.33, 0.11)
	No (n=76)	32/35 (91.4%)	36/41 (87.8%)	0.04 (-0.12, 0.20)
AK Stage	Stage-1 (n=22)	12/14 (85.7%)	8/8 (100%)	-0.14 (-0.43, 0.34)
	Stage-2 (n=87)	37/41 (90.2%)	40/46 (87.0%)	0.03 (-0.12, 0.18)
	Stage-3 (n=18)	7/11 (63.6%)	6/7 (85.7%)	-0.22 (-0.60, 0.26)
Age	< 25 years (n=30)	14/16 (87.5%)	14/14 (100%)	-0.13 (-0.38, 0.13)
	25-34 years (n=31)	16/18 (88.9%)	12/13 (92.3%)	-0.03 (-0.28, 0.26)
	35-45 years (n=32)	15/18 (83.3%)	12/14 (85.7%)	-0.02 (-0.30, 0.27)
	>=46 years (n=34)	11/14 (78.6%)	16/20 (80.0%)	-0.01 (-0.34, 0.28)

Table 40. Clinical resolution rate in patients with and without adjunctive treatments (FAS)

	0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL propamidine (N=61)	Total (N=127)
Patients with adjunctive treatment	N=30	N=25	N=55
Clinical resolution rate (%)	21 (70.0)	18 (72.0)	39 (70.9)
Fisher exact text for difference between treatments (p value)			1.0000
Patients without adjunctive treatment	N=36	N=36	N=72
Clinical resolution rate (%)	35 (97.2)	36 (100)	71 (98.6)
Fisher exact text for difference between treatments (p value)			1.0000
Adjunctive treatments were described in the protocol as: topical steroids or oral NSAIDs added or increased after baseline			

Relapses

No relapses occurred in the study during the follow-up period off therapy.

Table 41. Subjects with worsening of the condition (Study 043, ITT analysis set)

Rate of subjects with a worsening of the condition and following intensive care	0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/ml Propamidine (N=61)
Worsening of the condition	4 (6.1%)	0
No worsening of the condition	62 (93.9%)	61 (100.0%)

Source: Study 043/SI CSR, Table 14.3.8.1
Percentages are based on the number of subjects within each treatment group

Corneal inflammation

The changes in the individual components of corneal inflammation (corneal epithelial defects, ulceration, epithelial and corneal infiltrates, scarring) between baseline and end of the study were investigated. For corneal scarring and corneal ulceration see secondary endpoints.

Table 42. Corneal epithelial defects (study 043, ITT analysis set)

	0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=61)	Total (N=127)
Baseline			
Absent	19 (28.8%)	18 (29.5%)	37 (29.1%)
Present	47 (71.2%)	43 (70.5%)	90 (70.9%)
Missing	0	0	0
End-of-study visit			
Absent	56 (84.8%)	49 (80.3%)	105 (82.7%)
Present	8 (12.1%)	6 (9.8%)	14 (11.0%)
Missing	2 (3.0%)	6 (9.8%)	8 (6.3%)
McNemar's test p-value for difference from baseline within treatment group	<0.0001	<0.0001	<0.0001
Shift from baseline to End-of-study visit			
Absent to Absent	19 (28.8%)	16 (26.2%)	35 (27.6%)
Present to Absent	37 (56.1%)	33 (54.1%)	70 (55.1%)
Present to Present	8 (12.1%)	6 (9.8%)	14 (11.0%)
Missing at any of these visits	2 (3.0%)	6 (9.8%)	8 (6.3%)
Odds Ratio (95% CI) [1]	0.84 (0.26, 2.68)	-	-
p-value	0.769	-	-

Source: Study 043/SI CSR, Tables 14.2.4.1 and 14.2.4.2.
CI = confidence interval.
[1] Ratio comparison is based on logistic regression model using treatment group and baseline as predictors. Number of subjects included in the analysis are those with a baseline and an end of the study value (n=64 and n= 55, in 0.8 mg/mL polihexanide + placebo and 0.2 mg/mL polihexanide + 1.0 mg/mL propamidine, respectively).
The model is modelling the probability that the parameter is absent.

Table 43. Corneal epithelial opacity/infiltrates (Study 043, ITT analysis set)

	0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=61)	Total (N=127)
Baseline			
Absent	15 (22.7%)	17 (27.9%)	32 (25.2%)
Present	50 (75.8%)	44 (72.1%)	94 (74.0%)
Missing	1 (1.5%)	0	1 (0.8%)
End-of-study visit			
Absent	51 (77.3%)	45 (73.8%)	96 (75.6%)
Present	13 (19.7%)	10 (16.4%)	23 (18.1%)
Missing	2 (3.0%)	6 (9.8%)	8 (6.3%)
McNemar's test p-value for difference from baseline within treatment group	<0.0001	<0.0001	<0.0001
Shift from baseline to End-of-study visit			
Absent to Absent	11 (16.7%)	11 (18.0%)	22 (17.3%)
Absent to Present	2 (3.0%)	3 (4.9%)	5 (3.9%)
Present to Absent	39 (59.1%)	34 (55.7%)	73 (57.5%)
Present to Present	11 (16.7%)	7 (11.5%)	18 (14.2%)
Missing at any of these visits	3 (4.5%)	6 (9.8%)	9 (7.1%)
Odds Ratio (95% CI) [1]	0.86 (0.34, 2.15)		
p-value	0.743		

Sources: Study 043/SI CSR, Tables 14.2.4.5 and 14.2.4.6.
CI = confidence interval.
[1] Ratio comparison is based on logistic regression model using treatment group and baseline as predictors. Number of subjects included in the analysis are those with a baseline and an end of the study value (n=64 and n= 55, in 0.8 mg/mL polihexanide + placebo and 0.2 mg/mL polihexanide + 1.0 mg/mL propamidine, respectively). The model is modelling the probability that the parameter is absent.

Table 44. Corneal stromal opacity/infiltrates (Study 043, ITT analysis set)

	0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=61)	Total (N=127)
Baseline			
Absent	21 (31.8%)	23 (37.7%)	44 (34.6%)
Present	45 (68.2%)	38 (62.3%)	83 (65.4%)
Missing	0	0	0
End-of-study visit			
Absent	34 (51.5%)	33 (54.1%)	67 (52.8%)
Present	30 (45.5%)	22 (36.1%)	52 (40.9%)
Missing	2 (3.0%)	6 (9.8%)	8 (6.3%)
McNemar's test p-value for difference from baseline within treatment group	0.0039	0.0411	0.0005
Shift from baseline to End-of-study visit			
Absent to Absent	13 (19.7%)	13 (21.3%)	26 (20.5%)
Absent to Present	6 (9.1%)	9 (14.8%)	15 (11.8%)
Present to Absent	21 (31.8%)	20 (32.8%)	41 (32.3%)
Present to Present	24 (36.4%)	13 (21.3%)	37 (29.1%)
Missing at any of these visits	2 (3.0%)	6 (9.8%)	8 (6.3%)
Odds Ratio (95% CI) [1]	0.79 (0.38, 1.64)		
p-value	0.523		

Source: Study 043/SI CSR, Tables 14.2.4.7 and 14.2.4.8.
CI = confidence interval.
[1] Ratio comparison is based on logistic regression model using treatment group and baseline as predictors. Number of subjects included in the analysis are those with a baseline and an end of the study value (n=64 and n= 55, in 0.8 mg/mL polihexanide + placebo and 0.2 mg/mL polihexanide + 1.0 mg/mL propamidine, respectively). The model is modelling the probability that the parameter is absent.

Conjunctival inflammation

The changes in the individual components of conjunctival inflammation (conjunctival erythema, oedema, discharge, papillae and follicles) between baseline and end of the study were investigated.

Table 46. Conjunctival erythema (Study 043, ITT analysis set)

	0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=61)	Total (N=127)
Baseline			
Mild	30 (45.5%)	21 (34.4%)	51 (40.2%)
Moderate	24 (36.4%)	34 (55.7%)	58 (45.7%)
None	5 (7.6%)	1 (1.6%)	6 (4.7%)
Severe	7 (10.6%)	5 (8.2%)	12 (9.4%)
Missing	0	0	0
End-of-study visit			
Mild	6 (9.1%)	3 (4.9%)	9 (7.1%)
Moderate	3 (4.5%)	1 (1.6%)	4 (3.1%)
None	55 (83.3%)	50 (82.0%)	105 (82.7%)
Severe	0	1 (1.6%)	1 (0.8%)
Missing	2 (3.0%)	6 (9.8%)	8 (6.3%)
Paired T-test p-value for change from baseline within treatment group	<0.0001	<0.0001	<0.0001
Shift from baseline to End-of-study visit			
Mild to Mild	1 (1.5%)	1 (1.6%)	2 (1.6%)
Mild to None	28 (42.4%)	20 (32.8%)	48 (37.8%)
Moderate to Mild	3 (4.5%)	1 (1.6%)	4 (3.1%)
Moderate to Moderate	2 (3.0%)	1 (1.6%)	3 (2.4%)
Moderate to None	18 (27.3%)	28 (45.9%)	46 (36.2%)
Moderate to Severe	0	1 (1.6%)	1 (0.8%)
None to Moderate	1 (1.5%)	0	1 (0.8%)
None to None	4 (6.1%)	1 (1.6%)	5 (3.9%)
Severe to Mild	2 (3.0%)	1 (1.6%)	3 (2.4%)
Severe to None	5 (7.6%)	1 (1.6%)	6 (4.7%)
Missing at any of these visits	2 (3.0%)	6 (9.8%)	8 (6.3%)
Odds Ratio (95% CI) [1]	1.64 (0.61, 4.38)		
p-value	0.411		

Sources: Study 043/SI CSR, Tables 14.2.4.2.1 and 14.2.4.2.2.
CI = confidence interval.
[1] Ratio comparison is based on ordinal logistic regression model using treatment group and baseline as predictors. Number of subjects included in the analysis are those with a baseline and an end of the study value (n=64 and n= 55, in 0.8 mg/mL polihexanide + placebo and 0.2 mg/mL polihexanide + 1.0 mg/mL propamidine, respectively).
The model is modelling the probability that the parameter is decreasing.

Table 47. Conjunctival oedema (Study 043, ITT analysis set)

	0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=61)	Total (N=127)
Baseline			
Mild	16 (24.2%)	23 (37.7%)	39 (30.7%)
Moderate	11 (16.7%)	9 (14.8%)	20 (15.7%)
None	36 (54.5%)	27 (44.3%)	63 (49.6%)
Severe	3 (4.5%)	2 (3.3%)	5 (3.9%)
Missing	0	0	0
End-of-study visit			
Mild	1 (1.5%)	1 (1.6%)	2 (1.6%)
Moderate	2 (3.0%)	1 (1.6%)	3 (2.4%)
None	61 (92.4%)	53 (86.9%)	114 (89.8%)
Severe	0	0	0
Missing	2 (3.0%)	6 (9.8%)	8 (6.3%)
Paired T-test p-value for change from baseline within treatment group	<0.0001	<0.0001	<0.0001
Shift from baseline to End-of-study visit			
Mild to Mild	1 (1.5%)	1 (1.6%)	2 (1.6%)
Mild to None	14 (21.2%)	19 (31.1%)	33 (26.0%)
Moderate to Moderate	1 (1.5%)	0	1 (0.8%)
Moderate to None	10 (15.2%)	8 (13.1%)	18 (14.2%)
None to Moderate	1 (1.5%)	1 (1.6%)	2 (1.6%)
None to None	34 (51.5%)	24 (39.3%)	58 (45.7%)
Severe to None	3 (4.5%)	2 (3.3%)	5 (3.9%)
Missing at any of these visits	2 (3.0%)	6 (9.8%)	8 (6.3%)
Odds Ratio (95% CI) [1]	1.32 (0.21, 8.19)		
p-value	0.767		

Source: Study 043/SI CSR, Table 14.2.4.2.3 and 14.2.4.2.4.

CI = confidence interval.

[1] Ratio comparison is based on ordinal logistic regression model using treatment group and baseline as predictors. Number of subjects included in the analysis are those with a baseline and an end of the study value (n=64 and n= 55, in 0.8 mg/mL polihexanide + placebo and 0.2 mg/mL polihexanide + 1.0 mg/mL propamidine, respectively).

The model is modelling the probability that the parameter is decreasing.

Table 48. Conjunctival discharge (Study 043, ITT analysis set)

	0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=61)	Total (N=127)
Baseline			
Absent	58 (87.9%)	52 (85.2%)	110 (86.6%)
Present	7 (10.6%)	7 (11.5%)	14 (11.0%)
Missing	1 (1.5%)	2 (3.3%)	3 (2.4%)
End-of-study visit			
Absent	60 (90.9%)	53 (86.9%)	113 (89.0%)
Present	0	0	0
Missing	6 (9.1%)	8 (13.1%)	14 (11.0%)
McNemar's test p-value for difference from baseline within treatment group	<0.0001	<0.0001	<0.0001
Shift from baseline to End-of-study visit			
Absent to Absent	52 (78.8%)	45 (73.8%)	97 (76.4%)
Present to Absent	7 (10.6%)	6 (9.8%)	13 (10.2%)
Missing at any of these visits	7 (10.6%)	10 (16.4%)	17 (13.4%)
Odds Ratio (95% CI) [1]	0.86 (0.34, 2.15)		
p-value	0.743		

Source: Study 043/SI CSR, Tables 14.2.4.2.5 and 14.2.4.2.6.
CI = confidence interval.
[1] Ratio comparison is based on ordinal logistic regression model using treatment group and baseline as predictors. Number of subjects included in the analysis are those with a baseline and an end of the study value (n=64 and n= 55, in 0.8 mg/mL polihexanide + placebo and 0.2 mg/mL polihexanide + 1.0 mg/mL propamidine, respectively).
The model is modelling the probability that the parameter is absent.

Table 49. Conjunctival papillae (Study 043, ITT analysis set)

	0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=61)	Total (N=127)
Baseline			
Absent	44 (66.7%)	37 (60.7%)	81 (63.8%)
Present	20 (30.3%)	20 (32.8%)	40 (31.5%)
Missing	2 (3.0%)	4 (6.6%)	6 (4.7%)
End-of-study visit			
Absent	55 (83.3%)	46 (75.4%)	101 (79.5%)
Present	9 (13.6%)	9 (14.8%)	18 (14.2%)
Missing	2 (3.0%)	6 (9.8%)	8 (6.3%)
McNemar's test p-value for difference from baseline within treatment group	0.0116	0.0325	0.0009
Shift from baseline to End-of-study visit			
Absent to Absent	39 (59.1%)	32 (52.5%)	71 (55.9%)
Absent to Present	4 (6.1%)	3 (4.9%)	7 (5.5%)
Present to Absent	15 (22.7%)	11 (18.0%)	26 (20.5%)
Present to Present	4 (6.1%)	5 (8.2%)	9 (7.1%)
Missing at any of these visits	4 (6.1%)	10 (16.4%)	14 (11.0%)
Odds Ratio (95% CI) [1]	1.26 (0.42, 3.73)		
p-value	0.679		

Source: Study 043/SI CSR, Tables 14.2.4.2.7 and 14.2.4.2.8.
CI = confidence interval.
[1] Ratio comparison is based on ordinal logistic regression model using treatment group and baseline as predictors. Number of subjects included in the analysis are those with a baseline and an end of the study value (n=64 and n= 55, in 0.8 mg/mL polihexanide + placebo and 0.2 mg/mL polihexanide + 1.0 mg/mL propamidine, respectively).
The model is modelling the probability that the parameter is absent

Table 50. Conjunctival follicles (Study 043, ITT analysis set)

	0.8 mg/mL polihexanide + placebo (N=66)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=61)	Total (N=127)
Baseline			
Absent	60 (90.9%)	49 (80.3%)	109 (85.8%)
Present	5 (7.6%)	11 (18.0%)	16 (12.6%)
Missing	1 (1.5%)	1 (1.6%)	2 (1.6%)
End-of-study visit			
Absent	57 (86.4%)	49 (80.3%)	106 (83.5%)
Present	6 (9.1%)	6 (9.8%)	12 (9.4%)
Missing	3 (4.5%)	6 (9.8%)	9 (7.1%)
McNemar's test p-value for difference from baseline within treatment group	<0.0001	<0.0001	<0.0001
Shift from baseline to End-of-study visit			
Absent to Absent	54 (81.8%)	40 (65.6%)	94 (74.0%)
Absent to Present	3 (4.5%)	3 (4.9%)	6 (4.7%)
Present to Absent	3 (4.5%)	8 (13.1%)	11 (8.7%)
Present to Present	2 (3.0%)	3 (4.9%)	5 (3.9%)
Missing at any of these visits	4 (6.1%)	7 (11.5%)	11 (8.7%)
Odds Ratio (95% CI) [1]	0.99 (0.26, 3.81)		
p-value	0.990		

Source: Study 043/SI CSR, Tables 14.2.4.2.9 and 14.2.4.2.10.
CI = confidence interval
[1] Ratio comparison is based on ordinal logistic regression model using treatment group and baseline as predictors. Number of subjects included in the analysis are those with a baseline and an end of the study value (n=63 and n= 55, in 0.8 mg/mL polihexanide + placebo and 0.2 mg/mL polihexanide + 1.0 mg/mL propamidine, respectively).
The model is modelling the probability that the parameter is absent.

Additional ocular signs

Table 51. Lens (FAS)

Lens	0.8 mg/ml polihexanide + placebo (N=66)	0.2 mg/ml polihexanide + 1 mg/ml propamidine (N=61)	Total (N=127)
Baseline			
Normal	63 (95.5%)	58 (95.1%)	121 (95.3%)
Abnormal, NCS	2 (3.0%)	2 (3.3%)	4 (3.1%)
Abnormal, CS	0	0	0
Missing	1 (1.5%)	1 (1.6%)	2 (1.6%)
End-of-study visit			
Normal	57 (86.4%)	51 (83.6%)	108 (85.0%)
Abnormal, NCS	2 (3.0%)	1 (1.6%)	3 (2.4%)
Abnormal, CS	1 (1.5%)	0	1 (0.8%)
Missing	6 (9.1%)	9 (14.8%)	15 (11.8%)

CS = clinically significant, NCS = not clinically significant
Baseline is defined as the last valid evaluation prior to the first dose of study medication.
Percentages are based on the number of subjects within each treatment group
Program: \Subprogs\Tables\Lens.sas
Date and time program was run: 2022-03-09T08:28. Date and time analysis database was run: 2021-11-30T18:15

- **Summary of main efficacy results**

This submission includes 1 pivotal study (ODAK Phase 3, 043/SI). The following table summarises the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 53. Summary of efficacy for trial ODAK Phase 3, 043/SI

Title: Randomized, Assessor-Masked, Active-Controlled, Phase 3 Study to Evaluate Efficacy, Safety and Tolerability of 0.08% Polyhexamethylene Biguanide (polihexanide) Ophthalmic Solution in Comparison with 0.02% polihexanide + 0.1% Propamidine Combination Therapy in Subjects Affected by *Acanthamoeba* keratitis.]		
Study identifier	ODAK Phase 3 (043/SI) EudraCT Number: 2016-001823-30	
Design	Randomized, Assessor-Masked, Active-Controlled, Phase 3 Study	
	Duration of main phase:	Subjects were treated for a maximum of 1 year after randomization. Subjects meeting the criteria for clinical resolution at or before 12 months were followed up for 90 days before completing the study.
	Duration of Run-in phase:	Not applicable
	Duration of Extension phase:	Not applicable
Hypothesis	The study was designed as a superiority study with the possibility to test for non-inferiority if the superiority hypothesis was not met, according to the requirements of the guidance from the European Medicines Agency (EMA) (CPMP/EWP/482/99). **The primary hypothesis** was to test that the cure rate within 12 months (CRR_12) of subjects treated with 0.8 mg/ml polihexanide 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 0.2 mg/ml polihexanide + 1 mg/ml 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** were: • That adverse events, and those relating to toxicity in particular, are less with polihexanide 0.8 mg/ml monotherapy compared to the comparator. • That time to a cure is shorter in subjects receiving 0.8 mg/ml monotherapy compared to the comparator. Note: The estimated CRR_12 from the start of treatment is 67% for the conventional (0.2 mg/ml polihexanide + 1 mg/ml 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).	
Treatments groups	**Group 1:** 0.8 mg/ml polihexanide + placebo	1 drop of in the affected eye according to the following regimen: Day 0 to 5: 16 times a day at 1-hour intervals, daytime only, for 5 days Day 6 to 12: 8 times a day at 2-hour intervals, daytime only, for 7 days

		Day 13 to 19: 6 times a day at 3-hour intervals, daytime only, for 7 days From Day 20 onwards: 4 times a day at 4-hour intervals until resolution (maximum 12 months) Number of patients randomized = 69.
	Group 2: 0.2 mg/ml polihexanide + 1 mg/ml propamidine combination therapy	1 drop of in the affected eye according to the following regimen: Day 0 to 5: 16 times a day at 1-hour intervals, daytime only, for 5 days Day 6 to 12: 8 times a day at 2-hour intervals, daytime only, for 7 days Day 13 to 19: 6 times a day at 3-hour intervals, daytime only, for 7 days From Day 20 onwards: 4 times a day at 4-hour intervals until resolution (maximum 12 months) Number of patients randomized = 66.
Endpoints and definitions	**Primary** **efficacy endpoint**	Clinical resolution rate at 12 months (CRR_12) from randomization, defined as the percentage of subjects cured 30 days after discontinuing all study therapies, within 12 months of randomization
	Secondary **efficacy endpoints:**	Following secondary endpoints were assessed before the first study drug application (Day 0), at each ambulant visits during treatment period (on Days 7, 14, 21, 30 and every 30 days until resolution) and follow-up period (performed 30 and 90 days after end of treatment).
		Best corrected visual acuity (BCVA)
		Corneal scarring
		Ulceration severity
		Assessment of conjunctiva
		Lens and pupil test
		Anterior chamber inflammation
		EuroQol five-dimension scale (EQ-5D) questionnaire
		Visual functioning questionnaire 25 (VFQ25)
		Time to cure
Database lock	8 October 2021	
<u>Results and Analysis</u>		
Analysis description	**Primary Analysis**	

<table>
<tr><td>Analysis population and time point description</td><td colspan="3">Full Analysis Set (FAS): All subjects who were randomized and for whom the diagnosis of Acanthamoeba keratitis was confirmed and the primary efficacy variable was assessed. The FAS is used in all efficacy analyses. The last disease status assessment was used for disease status assessment.

Per Protocol Analysis Set (PPAS): The subset of subjects in the FAS for whom no major protocol deviation judged as having an impact on the primary efficacy analysis was reported or identified. Subjects assigned to the PPAS attended the 30- and 90-day follow-up visits. The last disease status assessment within 12 months of randomization was used for disease status assessment. For subjects with disease resolution at that last visit, the 30-day and 90-day follow-up assessments should confirm disease resolution. Otherwise, the disease was regarded as not having been resolved.

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

<table>
<tr><th></th><th>0.8 mg/ml polihexanide + placebo</th><th>0.2 mg/ml polihexanide + 1 mg/ml propamidine</th><th>All subjects</th></tr>
<tr><td>Full Analysis Set</td><td>66</td><td>61</td><td>127</td></tr>
<tr><td>Per Protocol Analysis Set</td><td>62</td><td>57</td><td>119</td></tr>
</table>

The primary efficacy assessment included the evaluation of CRR which was assessed within 12 months of randomization.</td></tr>
<tr><td>Descriptive statistics and estimate variability</td><td colspan="3">
<table>
<tr><td>Treatment group</td><td>0.8 mg/ml polihexanide + placebo</td><td>0.2 mg/ml polihexanide + 1 mg/ml propamidine</td></tr>
<tr><td colspan="3">Endpoint</td></tr>
<tr><td colspan="3">CRR_12 (FAS)</td></tr>
<tr><td>Resolved</td><td>56 (84.8%)</td><td>54 (88.5%)</td></tr>
<tr><td>Ratio (95% CI)</td><td>5.60
(2.86, 10.98)</td><td>7.71 (3.51, 16.95)</td></tr>
<tr><td colspan="3">CRR_12 (PPAS)</td></tr>
<tr><td>Resolved</td><td>54 (87.1%)</td><td>51 (89.5%)</td></tr>
<tr><td>Ratio (95% CI)</td><td>6.75
(3.21, 14.18)</td><td>8.50
(3.65, 19.81)</td></tr>
<tr><td></td><td></td><td></td></tr>
</table>
</td></tr>
<tr><td>Effect estimate per comparison</td><td>Endpoint</td><td colspan="2">Comparison Groups:
Group 1: 0.8 mg/ml polihexanide + placebo
Group 2: 0.2 mg/ml polihexanide + 1 mg/ml propamidine combination therapy</td></tr>
</table>

	CRR_12 (FAS)	Odds Ratio* (95% CI)	0.73 (0.26, 2.04) p=0.544
		Test for non-inferiority: Differences in proportion of CRR_12 (95% CI)	-0.04 (-0.16, 0.09)
		The lower limit of the 90% CI was above -0.2, the criteria for non- inferiority were met.	
	CRR_12 (PPAS)	Odds Ratio* (95% CI)	0.79 (0.26, 2.45) p=0.688
		Test for non-inferiority: Differences in proportion of CRR_12 (95% CI)	-0.02 (-0.15, 0.10)
		The lower limit of the 95% CI was above -0.2, the criteria for non- inferiority were met.	
Analysis description	**Secondary Analysis**		
Analysis population and time point description	See section on "primary analysis" above for the definitions of the FAS and the PPAS populations. All efficacy evaluations were based on the FAS. Secondary efficacy was assessed before the first study drug application (Day 0), at each ambulant visits during treatment period (on Days 7, 14, 21, 30 and every 30 days until resolution) and follow-up period (performed 30 and 90 days after end of treatment).		

Descriptive statistics and estimate variability

Secondary Endpoint	**0.8 mg/ml polihexanide + placebo**	**0.2 mg/ml polihexanide + 1 mg/ml propamidine**
BCVA		
Mean change from Baseline in Log MAR score (90% CI)	-0.25 (-0.32, -0.18)	-0.30 (-0.38, -0.22)
Assessment of Corneal Scarring		
Corneal scarring		
Shift from baseline to end-of-study: present to absent	0	1 (1.6%)
Corneal Epithelial Defects		
Shift from baseline to end-of-study: present to absent	37 (56.1%)	33 (54.1%)
Corneal Epithelial Opacity/Infiltrate		
Shift from baseline to end-of-study: present to absent	39 (59.1%)	34 (55.7%)
Corneal Stromal Opacity/Infiltrate		

	Shift from baseline to end-of-study: present to absent	21 (31.8%)	20 (32.8%)
	Corneal Ulceration		
	Shift from baseline to end-of-study: present to absent	16 (24.2%)	8 (13.1%)
	Assessment of conjunctiva		
	Erythema grade		
	Shift from baseline to end-of-study: Mild to none Moderate to none Severe to none	 28 (42.4%) 18 (27.3%) 5 (7.6%)	 20 (32.8%) 28 (45.9%) 1 (1.6%)
	Edema grade		
	Shift from baseline to end-of-study: Mild to none Moderate to none Severe to none	 14 (21.2%) 10 (15.2%) 3 (4.5%)	 19 (31.1%) 8 (13.1%) 2 (3.3%)
	Discharge		
	Shift from baseline to end-of-study: present to absent	7 (10.6%)	6 (9.8%)
	Papillae		
	Shift from baseline to end-of-study: present to absent	15 (22.7%)	11 (18.0%)
	Follicles		
	Shift from baseline to end-of-study: present to absent	3 (4.5%)	8 (13.1%)
	Lens and Pupil		
	Lens		
	Normal at Baseline	63 (95.5%)	58 (95.1%)
	Abnormal, NCS at Baseline	2 (3.0%)	2 (3.3%)
	Normal at end-of-study	57 (86.4%)	51 (83.6%)
	Abnormal, NCS at end-of-study	2 (3.0%)	1 (1.6%)
	Abnormal, CS at end-of-study	1 (1.5%)	0
	Pupil		
	Normal at Baseline	66 (100.0%)	60 (98.4%)
	Abnormal, NCS at Baseline	0	0
	Normal at end-of-study visit	62 (93.9%)	54 (88.5%)
	Abnormal, NCS at end-of-study	1 (1.5%)	1 (1.6%)
	Anterior chamber inflammation assessment		
	Shift from baseline to end-of-study: None to none Mild to none Moderate to none Severe to none	 49 (74.2%) 10 (15.2%) 1 (1.5%) 1 (1.5%)	 36 (59.0%) 10 (16.4%) 3 (4.9%) 1 (1.6%)
	EQ-5D-5L		
	EQ-5D-5L VAS score		

	Mean change from baseline to end-of-study (SD)	17.9 (19.6)	18.8 (20.0)
	EQ-5D-5L Mobility score		
	LS Mean at end-of study (90% CI)	1.12 (1.02, 1.23)	1.14 (1.03, 1.25)
	EQ-5D-5L Self-care dimensions		
	LS Mean at end-of study (90% CI)	1.04 (0.97, 1.10)	1.06 (0.99, 1.13)
	EQ-5D-5L Usual activities dimension		
	LS Mean at end-of study (90% CI)	1.18 (1.04, 1.32)	1.34 (1.19, 1.50)
	EQ-5D-5L Pain/Discomfort dimension		
	LS Mean at end-of study (90% CI)	1.34 (1.20, 1.48)	1.38 (1.23, 1.53)
	EQ-5D-5L Anxiety/ Depression dimension		
	LS Mean at end-of study (90% CI)	1.34 (1.22, 1.45)	1.32 (1.20, 1.45)
	VFQ-25 score		
	VFQ-25 Composite Score		
	Mean change from baseline to end-of-study (SD)	23.5 (19.4)	23.7 (19.7)
	General Health		
	Mean change from baseline to end-of-study (SD)	12.9 (27.8)	14.1 (24.4)
	General Vision		
	Mean change from baseline to end-of-study (SD)	24.7 (23.1)	23.6 (22.1)
	Time to cure		
	Median time to cure (days)	140	114
Effect estimate per comparison	**Secondary Endpoint**	**Comparison Groups**: **Group 1**: 0.8 mg/ml polihexanide + placebo **Group 2:** 0.2 mg/ml polihexanide + 1 mg/ml propamidine combination therapy	
	BCVA		
	Difference between groups (90% CI)[$]	0.05 (-0.06, 0.16) p=0.444	
	Assessment of Corneal Scarring		
	Corneal scarring		
	Odds Ratio* (95% CI)	1.14 (0.55, 2.36) p=0.734	
	Corneal Epithelial Defects		
	Odds Ratio* (95% CI)	0.84 (0.26, 2.68) p=0.769	
	Corneal Epithelial Opacity/Infiltrate		
	Odds Ratio* (95% CI)	0.86 (0.34, 2.15) p=0.743	
	Corneal Stromal Opacity/Infiltrate		

Odds Ratio* (95% CI)	0.79 (0.38, 1.64) p=0.523
Corneal Ulceration	
Odds Ratio* (95% CI)	1.09 (0.22, 5.47) p=0.917
Assessment of conjunctiva	
Erythema grade	
Odds Ratio** (95% CI)	1.64 (0.61, 4.38) p=0.411
Edema grade	
Odds Ratio** (95% CI)	1.32 (0.21, 8.19) p=0.767
Discharge	
Odds Ratio** (95% CI)	0.86 (0.34, 2.15) p=0.743
Papillae	
Odds Ratio* (95% CI)	1.26 (0.42, 3.73) p=0.679
Follicles	
Odds Ratio* (95% CI)	0.99 (0.26, 3.81) p=0.990
Lens and Pupil	
Lens	Not applicable, as no comparison between-treatment group was performed.
Pupil	Not applicable, as no comparison between-treatment group was performed.
Anterior chamber inflammation assessment	
Odds Ratio** (95% CI)	0.65 (0.14, 3.11) p=0.651
EQ-5D-5L	
EQ-5D-5L VAS score	
Difference in LS Mean (90% CI)[$]	-1.97 (-5.56, 1.63) p=0.366
EQ-5D-5L Mobility score	
Difference in LS Mean (90% CI)[$]	-0.02 (-0.17, 0.13) p=0.840
EQ-5D-5L Self-care dimensions	
Difference in LS Mean (90% CI)[$]	-0.02 (-0.12, 0.07) p=0.699
EQ-5D-5L Usual activities dimension	
Difference in LS Mean (90% CI)[$]	-0.17 (-0.37, 0.04) p=0.187
EQ-5D-5L Pain/Discomfort dimension	
Difference in LS Mean (90% CI)[$]	-0.04 (-0.24, 0.17)

		p=0.772
	EQ-5D-5L Anxiety/ Depression dimension	
	Difference in LS Mean (90% CI)[$]	0.01 (-0.15, 0.18) p=0.915
	VFQ-25 score	
	VFQ-25 Composite Score	
	p-value for change comparison[$]	0.6546
	General Health	
	p-value for change comparison[$]	0.2527
	General Vision	
	p-value for change comparison[$]	0.5772
	Time to cure	
	Hazard ratio (90% CI)[$$]	0.68 (0.49, 0.94) p=0.0480
Notes	A total of 7 subjects were not included in the FAS analysis and only included in the safety set to avoid any bias of incorrect evaluations of subjects not having *Acanthamoeba* keratitis. These 7 subjects have been randomized and did not have a confirmed diagnosis of *Acanthamoeba* keratitis because they had uncertain *in vivo* confocal microscopy (IVCM) findings and both culture and PCR were negative. The primary reasons for withdrawal from the study and discontinuation from the study treatment were balanced between the treatment groups, and among the Safety Analysis Set and PPAS. The distribution of putative risk factors affecting CRR_12 between groups was analysed. There was a reasonable equal distribution for each risk factor and no significant relationship was observed between these factors and CRR_12. Overall, the CRR_12 was highest (20/22; 90.9%) for subjects with Stage-I of *Acanthamoeba* keratitis at baseline compared to the CRR_12 for subjects with Stage-II (77/87; 88.5%) and Stage III (13/18; 72.2%) of *Acanthamoeba* keratitis.	

ANCOVA=analysis of covariance; CI = confidence interval; FAS=Full Analysis Set; LS = least squares; PPAS= Per Protocol Analysis Set; SD = standard deviation
*Result displays ratio comparison of (0.8 mg/ml polihexanide + placebo)/(0.2 mg/ml polihexanide + 1 mg/ml Propamidine) and is based on logistic regression model.
**Result displays proportional odds ratio for 0.8 mg/ml polihexanide + placebo and is based on ordinal logistic regression model using treatment group and baseline as predictors.
[$] Based on ANCOVA model.

[$$] Based on Cox proportional hazards regression model.

2.6.5.3. Clinical studies in special populations

No studies were conducted with specific focus on special populations.

2.6.5.4. Analysis performed across trials (pooled analyses and meta-analysis)

Not applicable.

2.6.5.5. Supportive studies

1. STUDY TITLE: Retrospective evaluation of the clinical management of patients affected by *Acanthamoeba* keratitis

DESIGN: Observational retrospective clinical study

Study centres: two (2) clinical sites in Italy and UK

Objectives: The study evaluated, retrospectively, the clinical outcome of patients affected by AK treated with polihexanide or other anti-amoebal drugs.

Methodology: The study analysed patients affected by *Acanthamoeba* keratitis who were treated in 2 clinical sites from 1997 to 2007. Eligible patients included subjects with a diagnosis of *Acanthamoeba* keratitis in at least one eye. In case of bilateral infection, the eye showing the worse clinical picture was evaluated or, in case of equal clinical signs, the right eye was considered. Starting from the visit where the *Acanthamoeba* keratitis diagnosis was made (screening visit), data from all control visits registered in the clinical records up to the final endpoint visit conducted at least one month after treatment(s) suspension were included in the retrospective evaluation. No specific procedures were required for the evaluation of patients. Data were collected from all available visits.

Number of patients: Clinical records from 100 patients were selected and evaluated.

Diagnosis and main inclusion/exclusion criteria:

Inclusion criteria:

1. Patients with a diagnosis of *Acanthamoeba* keratitis presenting at least one of the following conditions: 1) Positive culture for *Acanthamoeba*; 2) Positive histology for *Acanthamoeba* ;3) Positive smear for *Acanthamoeba*; 4) Patients with history of contact lens wear showing the specific clinical findings suggestive of *Acanthamoeba* keratitis such as presence of perineural infiltrates and/or ring infiltrates and/or keratitis with/or without disproportionate pain
2. Patients having post-treatment control visits
3. Any time delay for AK diagnosis
4. Concomitant presence of a bacterial, viral or fungal ocular infection.

Exclusion criteria: none

Criteria for evaluation

EFFICACY. The primary efficacy variable was the clinical resolution rate defined as the percentage of patients resulting cured one month after discontinuing all therapies. Other efficacy parameters were: visual acuity, rate of patients requiring surgery; time to resolution of corneal defect and time to resolution of ocular inflammation, duration of acanthamoebicidal treatment(s).

SAFETY. Incidence of adverse reactions.

Treatment

During the course of the disease, the majority of patients (n=85) received polihexanide for their treatment, either as monotherapy or in combination with other anti-amoebic drugs; only 15 patients did not receive polihexanide. Drugs used, other than polihexanide, were diamidines (propamidine or hexamidine), chlorhexidine, neomycin and itraconazole. A minority of patients (17%) were treated only with 1 drug (either polihexanide or chlorhexidine) whereas most of them were treated with a variety of combinations including two or three drugs. The most used combination included polihexanide and one diamidine (51%): hexamidine was the diamidine most used in Italy whereas propamidine was the first choice in UK.

Sixty-two patients received corticosteroids during the course of the disease. The two most common corticosteroids taken were dexamethasone and prednisolone.

Table 54. Treatment

PHMB alone /combination (n=85)		Other (n=15)	
PHMB alone	10 (10%)	Chlorexidine alone	7 (7%)
PHMB +diamidine	51 (51%)	Chlorexidine + diamidine	7 (7%)
PHMB + Itraconazole	1 (1%)	Diamidine + neomycin	1 (1%)
PHMB + chlorexidine+ diamidine	23 (23%)		

The protocol specified six categories for the administered therapy: polihexanide monotherapy, polihexanide + diamidine (propamidine or hexamidine), polihexanide + chlorhexidine, chlorhexidine + diamidine (propamidine or hexamidine), chlorhexidine monotherapy and other. The intention in the protocol was that patients would be assigned to one of these six groups based on the therapies administered. However, when the medications were reviewed at the end of the study by the Investigators, over a third of the patients were treated with a complex combination of drugs which were not clearly specified in the above list and as a result of this these patients would have fallen into the 'Other' category. In order to prevent the majority of patients being allocated to the 'Other' category, these patients were allocated to the treatment group which, in the opinion of the Investigators, was associated with the resolution of *Acanthamoeba* keratitis in the patient.

EFFICACY RESULTS: Most patients affected by *Acanthamoeba* keratitis were contact lens wearers and 85% of them received treatment with polihexanide as monotherapy or in combination with other drug.

Regardless of the drugs used, 86% of patients were cured with medical therapy whereas 14% of them required therapeutic keratoplasty. The most used treatment protocol included a combination of polihexanide and a diamidine (hexamidine or propamidine). The resolution rate was similar between groups. Subgroup analysis based on the investigator perception found that the highest resolution rate was obtained with monotherapy (polihexanide or chlorhexidine) whereas the lowest resolution rate was obtained with the use of a combination of polihexanide plus diamidines or more complex regimens. These results were not statistically significant.

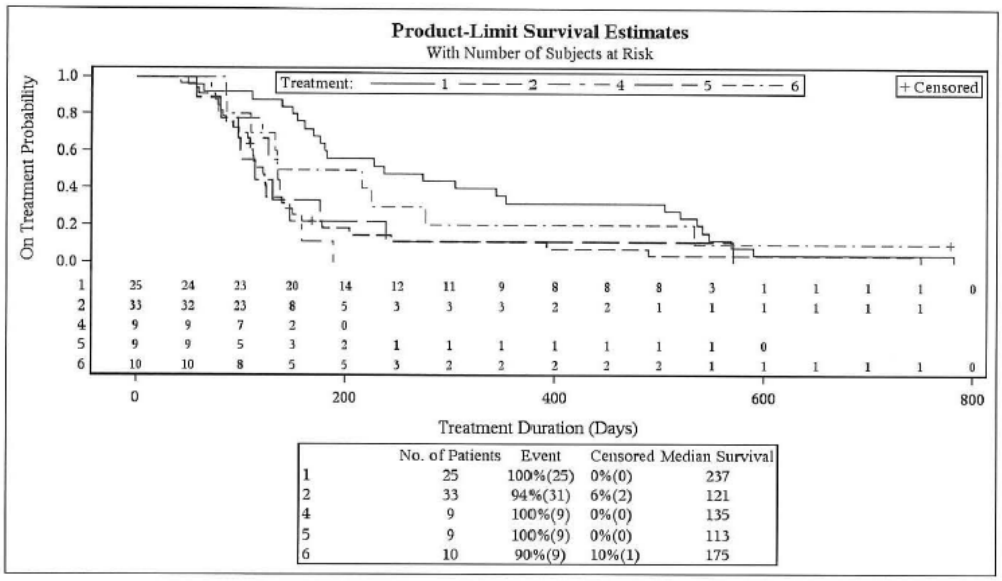
Table 55. Demography – Administered Therapy (All patients)

		PHMB monotherapy (N=26)	PHMB + diamidine (N=41)	PHMB + chlorhexidine (N=2)	Chlorhexidine + diamidine (N=10)	Chlorhexidine monotherapy (N=9)	Other (N=12)
AGE (YEARS)	N	26	41	2	10	9	12
	MEAN	34.7	30.6	54.5	31.5	37.7	33.3
	STD DEVIATION	13.18	13.72	6.36	9.79	16.87	10.92
	MINIMUM	19	14	50	20	23	19
	MEDIAN	32.5	28.0	54.5	28.5	31.0	33.0
	MAXIMUM	65	70	59	55	74	57
SEX (n %)	MALE	10 (38.46)	16 (39.02)	0 (0.00)	2 (20.00)	4 (44.44)	6 (50.00)
	FEMALE	16 (61.54)	25 (60.98)	2 (100.00)	8 (80.00)	5 (55.56)	6 (50.00)
RACE (n %)	CAUCASIAN	2 (7.69)	31 (75.61)	2 (100.00)	10 (100.00)	1 (11.11)	5 (41.67)
	OTHER	4 (15.38)	5 (12.20)	0 (0.00)	0 (0.00)	1 (11.11)	1 (8.33)
	UNKNOWN	20 (76.92)	5 (12.20)	0 (0.00)	0 (0.00)	7 (77.78)	6 (50.00)

Table 56. Clinical resolution rate (Subgroup analysis)

	PHMB Monotherapy N (%)	PHMB+ Diamidine N (%)	Chlorhexidine+ Diamidine N (%)	PHMB+ Chlorhexidine N (%)	Chlorhexidine Monotherapy N (%)	Other N (%)	Overall N (%)
Cured(total)	25 (96.15)	33 (80.5)	9 (90.0)	0 (0)	9 (100)	10 (83.3)	86 (86.0)
Not cured	1 (3.85)	8 (19.5)	1 (10.0)	2 (100)	0 (0)	2 (16.7)	14 (14.0)
Total	**26**	**41**	**10**	**2**	**9**	**12**	**100**

Figure 7. Duration of Acanthamoebicidal Treatment – Administered Therapy – Kaplan-Meier Plot (All patients)



	No. of Patients	Event	Censored	Median Survival
1	25	100%(25)	0%(0)	237
2	33	94%(31)	6%(2)	121
4	9	100%(9)	0%(0)	135
5	9	100%(9)	0%(0)	113
6	10	90%(9)	10%(1)	175

Treatment key: 1=PHMB monotherapy, 2=PHMB+diamidine, 4=Chlorhexidine+diamidine, 5=Chlorhexidine montherapy, 6=Other. Duration of Acanthamoebicidal Treatment is calculated as (date of last treatment)-(earliest date of acanthamoebicidal treatment)+1. Where applicable, data have been left censored using the date of diagnosis (date of symptom onset+time delay to diagnosis). Program: Figure2.sas. Supported by Listing 12.

Table 57. Corticosteroids – Administered Therapy (all patients)

Medication Name	PHMB monotherapy (N=26)	PHMB + diamidine (N=41)	PHMB + chlorhexidine (N=2)	Chlorhexidine + diamidine (N=10)	Chlorhexidine monotherapy (N=9)	Other (N=12)
TOTAL NUMBER (%) OF PATIENTS TAKING CORTICOSTEROIDS	24 (92.31)	20 (48.78)	0 (0.00)	3 (30.00)	6 (66.67)	9 (75.00)
BETAMETHASONE	0 (0.00)	1 (2.44)	0 (0.00)	0 (0.00)	0 (0.00)	1 (8.33)
BETAMETHASONE 0.1%	1 (3.85)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (16.67)
BETAMETHASONE 1% OINTMENT	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (8.33)
BETAMETHASONE OINTMENT	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (8.33)
BETAMETHASONE/CHLORAMPHENICOL	0 (0.00)	2 (4.88)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
DEXAMETHASONE	5 (19.23)	6 (14.63)	0 (0.00)	3 (30.00)	0 (0.00)	1 (8.33)
DEXAMETHASONE 0.1%	16 (61.54)	4 (9.76)	0 (0.00)	0 (0.00)	4 (44.44)	8 (66.67)
DEXAMETHASONE 0.15%	0 (0.00)	0 (0.00)	0 (0.00)	1 (10.00)	0 (0.00)	0 (0.00)

If a patient took a medication more than once, the patient has only been counted once within each category (ante-diagnosis, post-diagnosis and other). Corticosteroids include the following medications: Dexamethasone, Betamethasone, Metylprednisolone, Prednisolone, Prednisone and Fluorometholone. Corticosteroids are sorted alphabetically. All other medications have been listed only. The above table shows the number (%) of patients in each group taking various corticosteroids at some point. For example, for patients in the PHMB monotherapy group, the corticosteoids may have been taken before their first dose of PHMB but this is not reflected in the table. Program: Table21.sas. Supported by Listing 12.

Table 58. Duration of Acanthamocbicidal Treatment – Administered Therapy Wilcoxon Test (All patients)

		PHMB monotherapy (N=26)	PHMB + diamidine (N=41)	Chlorhexidine + diamidine (N=10)	Chlorhexidine monotherapy (N=9)	Other (N=12)	
DURATION OF ACANTHAMOEBICIDAL TREATMENT (DAYS):	25TH QUARTILE	161	92	120	97	109	
	MEDIAN	237	121	135	113	175	
	75TH QUARTILE	519	158	147	176	277	
	P-VALUE FROM WILCOXON TEST						0.0027

Duration of Acanthamoebicidal Treatment is calculated as (date of last treatment)-(earliest date when antiamebic drugs were used)+1.
Where applicable, data have been left censored using the date of diagnosis (date of symptom onset+time delay to diagnosis).
PHMB + chlorhexidine has not been included in the above table since neither patient in this group achieved clinical resolution.
Program: Table23.sas. Supported by Listing 12.

Table 59. Polihexanide Therapy Administration (All patients)

		PHMB (Alone/Combination) (N=85)
STARTING DOSE (DROPS/DAY):	N	85
	MEAN	17.3
	STD DEVIATION	6.12
	MINIMUM	4
	MEDIAN	16.0
	MAXIMUM	24

Figure 8. Duration of treatment (days): Polihexanide vs. other treatments (Kaplan Meier Plot)

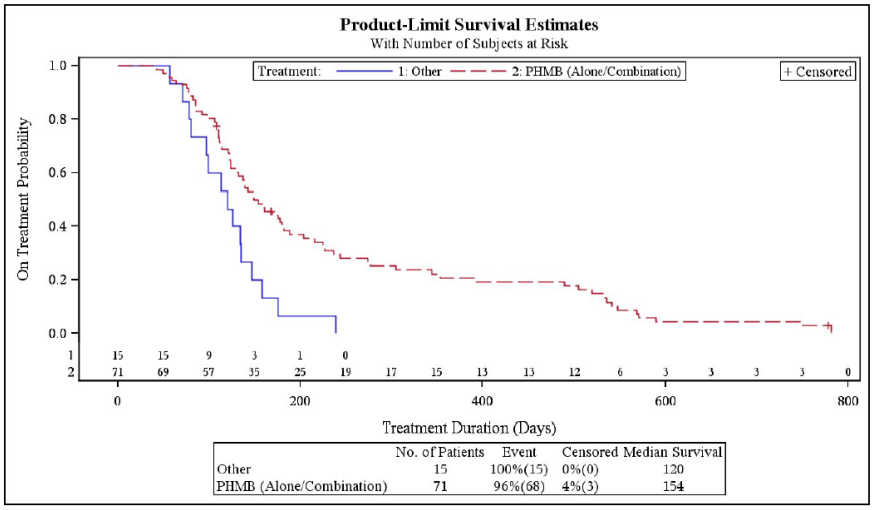


Table 60. Time-to-resolution of ocular inflammation (days)

	PHMB (Alone/combination) N =85	Other treatments N =15	Overall N =100
Mean(SD)	20.5 (23.3)	35.0 (52.0)	21.1 (24.6)
Median	15	8	15
Range	2-139	2-95	2-139

Table 61. Clinical Resolution (all Patients)

		PHMB (Alone/Combination) (N=85) n (%)	**Other (N=15) n (%)**	**Overall (N=100) n (%)**
WAS CLINICAL RESOLUTION OBTAINED?	YES	71 (83.53)	15 (100.00)	86 (86.00)
	NO	14 (16.47)	0 (0.00)	14 (14.00)

P-VALUE FROM CMH TEST	0.0888
P-VALUE FROM FISHER'S EXACT TEST	0.1202

All tests are two sided with a significance level of 0.05. The table contains information on clinical resolution at the patient's last visit only. Program: Table7.sas. Supported by Listing 8.

Table 62. Polihexanide starting dose

	N (%)
4 drops/day	4 (4.71)
6 drops /day	3 (3.53)
8 drops /day	7 (8.24)
12 drops /day	2 (2.35)
16 drops /day	35 (41.18)
20 drops /day	5 (5.88)
24 drops /day	29 (34.12)

Table 63. Comparison of cure rate within 12 months of initiating AK treatment (Papa et al. 2020; Table 2)

	Primary outcome for medical cure rate comparisons within 12 months of starting AAT for groups defined by baseline AAT compared with PHMB monotherapy						Outcomes for patients not achieving a medical cure at 12 months without surgery	
			Unadjusted		Adjusted for confounding*			
Baseline AAT	Cure	% cure (95% CI)	% cure ratio† (95% CI)	P value‡	% cure ratio† (95% CI)	P value‡	Cure rate at 12 months for medical therapy with surgery n (%)	Failure to cure at 12 months n (%)
#2 PHMB monotherapy (Referent)	29/50	58.00 (43.21 to 71.81)	1.00 (Referent)		1.00 (Referent)		8/50 (16.00)	13/50 (26.00)
#1 PHMB, diamidine	70/114	61.40 (51.83 to 70.37)	1.06 (0.80 to 1.40)	0.687	1.00 (0.78 to 1.29)	0.999	13/114 (11.40)	31/114 (27.19)
#3 Diamidine monotherapy	15/25	60.00 (38.67 to 78.87)	1.03 (0.69 to 1.54)	0.868	1.07 (0.79 to 1.45)	0.642	6/25 (24.00)	4/25 (16.00)
#7 Other AAT§	24/38	63.16 (46.00 to 78.19)	1.09 (0.78 to 1.53)	0.623	1.09 (0.79 to 1.50)	0.602	9/38 (23.68)	5/38 (13.16)
Totals	138/227	60.79 (54.11 to 67.19)		0.817¶		0.528¶	36/227 (15.86)	53/227 (23.35)
#1+#3+#7 Combined	109/177	61.58 (53.99 to 68.78)	1.06 (0.82 to 1.38)	0.656	1.03 (0.81 to 1.31)	0.804	28/177 (15.82)	40/177 (22.60)

2. STUDY TITLE: "Untreated" cases of Acanthamoeba keratitis: Systematic literature review report

DESIGN: Systematic literature review

Objective: A systematic literature search was conducted to identify published data about clinical outcomes in "untreated" cases of Acanthamoeba keratitis. 'Untreated' was defined as not receiving a treatment with an established and clinically proven anti-amoebic activity as stated by the CDC (polihexanide, chlorhexidine, propamidine, hexamidine).

Methods: Eligible studies were clinical studies, published between 1970-1995 inclusive, with patients with a confirmed diagnosis of Acanthamoeba keratitis who were "untreated" for whom a clinical outcome (cure without surgery; keratoplasty; enucleation) was reported. Database searches conducted on 27th November and 2nd December 2023 (PubMed; Cochrane Database of Systematic Reviews; Prospero International Prospective Register of Systematic Reviews; Cochrane Central Register of Controlled Trials; ClinicalTrials.gov) were supplemented with references from a previous targeted literature review and citation chasing. Screening was conducted by two independent reviewers. One reviewer rated the certainty of the overall body of evidence using the GRADE framework. The proportion [95% confidence interval adjusted for study-level clustering] of patients experiencing each outcome is presented.

Results: There were 37 eligible studies (56 patients in total), all of which were observational studies. The patients ranged between 13 and 71 years of age (mean = 34.9 years) and 50.0% were male. Most cases (n = 31; 55.4%) originated in the USA, with 10 (17.9%) from Europe. Most patients were administered corticosteroids (85.7%), antibiotics (82.1%), and/or antivirals (75.0%).

Table 64: Summarised characteristics of 56 patients with "untreated" AK (putative placebo).

Characteristic	Mean ± SD or Count (%)
Age, years[a]	34.9 ± 15.5 (range = 13 – 71)
Sex	
Men	28 (50.0%)
Women	26 (46.4%)
Missing	2 (3.6%)
Country where AK originated	
USA	31 (55.4%)
India	6 (10.7%)
Japan	5 (8.9%)
Australia	3 (5.4%)
Germany	3 (5.4%)
Denmark	2 (3.6%)
England	2 (3.6%)
Netherlands	2 (3.6%)
Belgium	1 (1.8%)
Philippines	1 (1.8%)
Concomitant medications by class	
Corticosteroids	48 (85.7%)
Antibiotics	46 (82.1%)
Antivirals	42 (75.0%)
Antifungals	30 (53.6%)
Mydriatrics	24 (42.9%)
Cycloplegics	8 (14.3%)
Antiglaucomas	6 (10.7%)
Beta-blockers	4 (7.1%)
NSAIDs	4 (7.1%)

Abbreviations: AK, Acanthamoeba keratitis; SD, Standard Deviation.
[a] Two missing values

Outcomes: GRADE quality of evidence: Low.

Overall, 11/56 patients (19.6%) were cured without medical or surgical intervention. An additional outcome was noted in the eligible articles that was not in the SLR protocol: cure with minor surgery. This occurred in two studies where a minor surgical procedure (debridement) proceeded cure. This procedure is not widely performed. It is made very extensive to remove as much infected corneal epithelium as possible, therefore patients who receive this procedure cannot be classed as cured without surgery nor did they have keratoplasty or enucleation. However, these cases were retained in the dataset for completeness as they met the eligibility criteria around being "untreated". There were 4 (7.1%) patients who were cured with minor surgery, 38 (67.9%) who required keratoplasty, and 4 (7.1%) who required enucleation.

Table 65: Outcomes of 56 patients with "untreated" AK (putative placebo).

Outcome	N analysed	N (%) with outcome	Proportion (95% CI)
Cured without surgery	56	11 (19.6%)	0.20 (0.08, 0.40)
Cured without surgery and with good vision[a]	50	1 (2.0%)	0.02 (0.00, 0.14)
Cured with minor surgery	56	4 (7.1%)	0.07 (0.01, 0.29)
Keratoplasty[b]	56	38 (67.9%)	0.68 (0.48, 0.83)
Enucleation or eye excision[b]	56	4 (7.1%)	0.07 (0.03, 0.18)

[a] Based on a final BCVA ≥20/40 (equivalent to ≥6/12 or ≤0.3). N analysed is lower for these analyses because final BCVA was not reported in some studies.
[b] One patient had a keratoplasty then an enucleation and so is included in both outcomes.

Conclusions: Overall, it was estimated that 20% (8%, 40%) of "untreated" patients (putative placebo) with Acanthamoeba keratitis were cured without surgery. The main limitation of the review was that it relies heavily on case reports and case series, which are subject to inherent bias, and require caution when interpreting the findings. However, evidence suggests that up until 1985, all diagnosed cases of Acanthamoeba keratitis were published, regardless of outcome or severity. Therefore, the cases

reports published up until 1985 are likely to be less prone to publication bias than those published after this date.

2.6.6. Discussion on clinical efficacy

Design and conduct of clinical studies

Polihexanide 0.8 mg/ml is intended to be applied as eye drops for the treatment of *Acanthamoeba* keratitis (AK). The product was granted an orphan designation in 2007 (EU/3/07/498). No treatment is licensed for the treatment of *Acanthamoeba* keratitis in the EEA.

Two prospectively planned clinical studies were conducted, study 042/SI (042) for dose selection based on the safety profile of three doses of polihexanide (i.e. 0.2, 0.6 and 0.8 mg/ml) in healthy volunteers and study 043/SI (043) to assess efficacy and safety compared to an unlicensed active comparator chosen to be a combination of polihexanide 0.2 mg/ml and propamidine 1 mg/ml in AK patients; the latter is the single pivotal trial supporting this application. In the context of the orphan nature of the disease, it is considered acceptable to base this MAA on a single pivotal trial.

To evaluate the efficacy and safety of current treatments of AK, an observational retrospective evaluation of the clinical management of patients affected by *Acanthamoeba* keratitis was performed for two clinical study sites in Italy and the UK from 1997 to 2007 (study 038/SI). The collection of data covered 10-20 years before start of the pivotal study in 2017 and whether the treatments used are relevant/generalisable to current EU practises in the continued absence of licensed regimens is questionable. Clinical records from 100 patients were selected and evaluated. The protocol specified six categories for the administered therapy: polihexanide monotherapy, polihexanide + diamidine (propamidine or hexamidine), polihexanide + chlorhexidine, chlorhexidine + diamidine (propamidine or hexamidine), chlorhexidine monotherapy and other. The applied dose and regimen of all treatments is unclear. The intention in the protocol was that patients would be assigned to one of these defined treatment groups based on the therapies administered. However, when the medications were reviewed at the end of the study by the investigators, over a third of the patients were treated with a complex combination of drugs which were not clearly specified. These patients were allocated to the treatment group which, in the opinion of the investigators, was associated with the resolution of *Acanthamoeba* keratitis in the patient. During the course of the disease, the majority of patients (n=85) received polihexanide for their treatment, either as monotherapy or in combination with other anti-amoebic drugs; only 15 patients did not receive polihexanide. Drugs used, other than polihexanide, were diamidines (propamidine or hexamidine), chlorhexidine, neomycin and itraconazole. A minority of patients (17%) were treated only with 1 drug (either polihexanide or chlorhexidine) whereas most of them were treated with a variety of combinations including two or three drugs. The most used combination included polihexanide and one diamidine (51%): hexamidine was the diamidine most used in Italy whereas propamidine was the first choice in UK. Sixty-two patients received corticosteroids during the course of the disease. Overall, this retrospective study does not provide clear support for the selected comparative regimen. However, at a Discussion Meeting during SA, it was agreed that the pragmatic way forward was to use polihexanide plus propamidine based on identification of a mutually acceptable control regimen with collective investigators that reflects current management approaches. The dataset from study 038/SI was later further expanded to 227 patients from Moorfield's Eye Hospital covering a time period from 1991 to 2012 and was published as Papa et al. 2020. Although the decision for developing a treatment for AK and selection of the candidate to be developed is up to the Applicant, it was noted that in a recently published retrospective analysis of 110 subjects with AK at one clinical site (Scruggs et al. 2022) chlorhexidine (0.02%) was the most used substance to treat AK. It was further noted that study 038/SI does suggest that polihexanide monotherapy is unlikely to be superior to a control regimen of polihexanide plus propamidine. There is also some evidence from

non-clinical studies that questions the usefulness of polihexanide for treatment of AK as some literature references (i.e. Narasimhan et al. 2002, Sunada et al. 2014 and Ruddell and Easty 1995) represent non-clinical studies in which the therapeutic efficacy of polihexanide against *Acanthamoeba* keratitis was either lacking, or clearly worse than applied comparator treatments. Biguanides, when used in combination with diamidines, were shown to have a synergistic effect (Dart JKG, Saw VPJ, Kilvington S. 2009;148:487–499. Hay J, Kirkness CM, Seal DV, et al. 1994). Of note, the effectiveness of all current unlicensed treatment options is unclear as none has been studied in a placebo controlled randomised clinical trial. Nevertheless, randomisation to placebo was/is considered unethical. This in turn already assumes some efficacy (at least above a potential placebo treatment) of the off-label anti-amoebic therapy that is currently used in clinical practice, including polihexanide. Thus, the use of polihexanide as treatment option can be followed, but superiority over a treatment option with similar MoA appears difficult to establish.

Comparator

Study 043 was initially planned to demonstrate superiority of the monotherapy treatment (polihexanide 0.8mg/ml + placebo; in this report sometimes referred to just polihexanide 0.8mg/ml) over the unlicensed combination therapy (polihexanide 0.2 mg/ml and propamidine 1 mg/ml), but included the option to switch to non-inferiority as per guideline (CPMP/EWP/482/99). Principally this approach appears acceptable. However, the applied non-inferiority margin (i.e. 0.2) cannot be justified on clinical or on statistical grounds. It is acknowledged that NI margins are difficult to define in a situation without licensed treatment (or at least well controlled trials). No RCT data of the (unlicensed) reference treatment compared to placebo is available and no indirect superiority (of the study drug over placebo) can therefore be established using this control arm. Therefore, statistical justification of the non-inferiority margin (i.e. ensuring superiority to "putative placebo") is hardly possible. Shortcomings of the non-inferiority approach as well as the chosen margin were also critically discussed during scientific advice, including a DM (see EMEA/H/SA/1064/2/2014/PA/III and EMA/CHMP/SAWP/401053/2016). Superiority to alternative treatment options with comparable/same mechanism of action in a well-managed CT setting appears also difficult to establish.

Considering that the active arm as applied in trial 043 contained a medicinal product manufactured to GMP quality, immediate availability and a standardized treatment protocol, it could not be considered a control representative of current practice.

Thus, an indirect comparison to historical reports of "untreated" AK patients appears to be a valid strategy to establish a treatment benefit.

After study completion and during assessment for authorisation, the Applicant decided to establish an absolute benefit-risk compared to an external control group of "untreated" patients based on a systematic screening of available literature regarding cases of "untreated" AK. The approach to establish an absolute treatment benefit via comparison of the Akantior arm in study 043 to historical control data, as for a single arm trial (SAT), is acknowledged. The treatment effect of interest is defined as the comparison (contrast) of the summary measure under the experimental treatment to the summary measure under the alternative of the trial population not being treated with the experimental treatment (counterfactual). For a SAT, the primary endpoint must also be able to isolate treatment effects, i.e. it is required that the primary endpoint is such that it is known that observations of the desired outcome would occur only to a negligible extent (in number of patients or size of the effect) in the absence of an active treatment. There can also be residual uncertainty about which outcomes are truly impossible without treatment, but there must be qualitative reasoning that leaves no doubt about the causal relationship between the treatment and outcome measured by the endpoint. When the treatment effect is clinically dramatic, occurs rapidly following treatment, and is unlikely to have occurred spontaneously, this may be sufficient to consider that isolation of the treatment effect

as well as clinical relevance are demonstrated (Reflection paper on establishing efficacy based on single arm trials submitted as pivotal evidence in a marketing authorisation, EMA/CHMP/564424/2021). The lack of randomization and blinding, and the resultant problems with lack of assurance of comparability of test group and control group, make the possibility of substantial bias inherent in this design and impossible to quantitate. The inability to control bias restricts use of the external control design to situations in which the effect of treatment is dramatic and the usual course of the disease highly predictable (ICH E10).

The retrospective systematic literature review (SLR) that was conducted to identify suitable reports of "untreated" patients appears appropriate, but still relies heavily on case reports and case series. 'Untreated' was defined as not receiving a treatment with an established and clinically proven anti-amoebic activity as stated by the CDC (polihexanide, chlorhexidine, propamidine, hexamidine). Identified patients were mostly treated with antibiotics, antifungals, antivirals and corticosteroids (also cycloplegics and mydriatrics are listed and in rare cases NSAID, anti-glaucomas and beta-blockers were used), which can indeed be considered of minor effectiveness against the underlying *Acanthamoeba* infection. Due to the assumed minor effectiveness of applied treatments, the estimation of the clinical resolution rate without surgery of "untreated" patients is considered rather conservative. There were 37 eligible studies identified (56 patients in total), all of which were observational studies. The target date range (1970-1995) included some very old publications which were not necessarily indexed in the databases that were searched. In the specific case of *Acanthamoeba* keratitis it seems unlikely that constancy on measures of disease resolution (at the time of considered reports compared to the time of study 043) is a major issue, but this cannot be dismissed. The GRADE assessment concluded a low certainty of evidence for the SLR based on the possible inherent bias, i.e. selection bias, information bias, time-related bias (Reflection paper on use of real-world data in non-interventional studies to generate real-world evidence, EMA/CHMP/150527/2024).

Study drug batches

The applicant confirmed that all batches used in clinical trials 042/SI and 043/SI were produced with the API supplied by Supplier X. Study batches were manufactured by Sifi SpA (3 batches per treatment concentration) or Rommelag (1 batch per treatment concentration). However, no information regarding the DP from Rommelag is provided in Module 3. The batches manufactured by Rommelag were used in only 15 patients, XC146 (polihexanide 0.8mg/ml) was used for 8 patients and XC145 (polihexanide 0.2mg/ml) was used in 7 patients. The quality of batches was confirmed by Sifi before use in the clinical phase 3 study. Upon request the Applicant also confirmed that batches from Rommelag were manufactured under GMP conditions. Furthermore, for 47 patients a batch resupply was required, for 27 patients treated with the combination therapy and for 20 patients treated with the monotherapy, but resupply of drug batches was not associated with any dose delay. Temporal delay in diagnosis/treatment of AK affected n=48 patients for 0-1 day, n=34 patients for 2-11 days and n=45 patients for 12-145 days.

Importantly, the DP used in clinical trials was produced with the DS supplied by Supplier X, whereas the commercial DP is intended to be based on the DS supplied by Supplier Y. Thus, no clinical data were generated in the RCT with the intended commercial DP. Based on quality data, small differences exist between the DP used for clinical trials and the intended commercial DP . Similarity cannot be further investigated on quality level, due to the absence of available DP supplied by Supplier X and hence remains partly undetermined. Thus, similarity of the clinical trial DP Supplier X and the intended commercial DP Supplier Y cannot be concluded on quality level (see Quality section for more details). Non-clinical bridging studies show that differences in some quality attributes (e.g. molecular weight of the polymers, biguanide content) between Supplier X and Supplier Y DP batches do not necessarily impact the *in vitro* anti-amoebal and anti-cystal efficacy as well as the *in vitro* safety (in terms of local tolerance and irritating potential) of polihexanide. Still, uncertainty remains because a.) the Supplier X

batches used for the *in vitro* bridging studies are not identical with the batches used in the original non-clinical and in the clinical studies, and because b.) an *in vitro* bridge might not necessarily cover the *in vivo* complexity of polihexanide in the treatment of *Acanthamoeba* keratitis in patients. Notably, the DP with DS from Supplier Y shows more consistent drug product quality as such. As Supplier Y is the intended DS for commercial use, this aspect is reassuring. Furthermore, it is reiterated that the Applicant has demonstrated that polihexanide with variable quality attributes, still shows comparable efficacy and tolerability in the *in vitro* setup. This observation is likely to be attributed to the non-specific mechanism of action, which might not depend on observed variations in biguanide concentration and molecular weight. Also, relevant adverse safety findings on non-clinical level were only observed for ten times higher concentrations then intended for clinical use, which appears reassuring regarding the expected safety on clinical level with observed variations in quality attributes. The clinical safety profile of Akantior appears rather tolerable, considering the severity of the disease (see discussion on safety). Furthermore, Akantior is to be used as local application only and systemic exposure appears negligible.

The applicant reported that 14 cases of AK that were followed in a currently running compassionate use programme of Akantior were all treated with the DS from Supplier Y and >90% were considered as resolved (Di Zazzo et al 2024; Franch et al 2024, submitted for publication). Of note, the compassionate use programme currently (May 2024) enrolled 155 patients, but only the resolution results of these 14 cases were provided so far, because other data were not publicly available yet. No thorough assessment of these data is possible in the scope of this submission. Although limited, these CUP data provide some reassurance. Based on the totality of data, it is concluded that the use of the DS from Supplier Y for the commercial DP (instead of the DS from and Supplier X employed for clinical studies) does not crucially impact the expected efficacy and safety profile of Akantior.

Study population and treatment

The included patient population in study 043 as defined by inclusion and exclusion criteria, specifically with regards to the diagnosis of AK, appears adequate. The treatment of subjects from 12 years of age, as intended by the Applicant, does not appear critical from the efficacy perspective as no deviations in the efficacy profile are expected between adolescent and adult subjects. The infectious disease is the same and adolescent eyes are not anatomically nor functionally different from adult eyes in matters of relevance to the disease to be treated. Extrapolation from the older population to patients in adolescence is therefore considered acceptable. Still, the limited number of subjects 12-18 years included in study 043 (n=3) is noted.

The chosen dose level of the monotherapy is based on the hypothesis that concentrations of polihexanide above 0.2 mg/ml may lead to better penetration of the cornea and this might reduce treatment failures. No data were provided that would support this assumption. Also, the dose-response relationship of polihexanide from 2-8 mg/ml is not convincingly demonstrated (see non-clinical AR). Thus, it remains unclear whether higher concentrations indeed penetrate deeper into the cornea as claimed by the Applicant. Currently no clinical data are available that would demonstrate that lower doses of polihexanide, as applied in current clinical practice, perform worse for the treatment of AK, especially when combined with diamidines. The dose of 0.8 mg/ml was ultimately chosen as it proved to be the highest tolerated dose tested in the Phase I trial. Still, the rationale for the choice of the higher dose of polihexanide is not convincingly demonstrated, as tolerability alone is not sufficient to conclude an added treatment benefit. It is highlighted that the choice for dose and dosing regimen of the investigational product is in the remit of the Applicant. However, uncertainty regarding the proposed benefit of the higher dose of polihexanide compared to usually administered lower doses remains as no evidence of clinical benefit over lower doses was established.

The same dose regimen was used for both study arms of the pivotal trial 043 and efficacy can only be evaluated for the chosen dose level and regimen. It is unclear whether the dosing regimen chosen for the investigational product was optimal for the comparator and whether a different dosing schedule for polihexanide or the combination therapy might be beneficial in terms of treatment outcome. The choice can be followed with respect to blinding of study treatments. In the clinical study setup two study drugs had to be applied for the investigational treatment arm with one being placebo due to blinding, whereas only one drug will have to be applied after potential licensure. Due to the potential of study drug dilution upon two subsequent ocular applications, the Applicant clarified that a 5-minute interval was followed between eye drop applications, which is considered sufficient to reduce a possible wash-out effect.

Only the right eye (or the worst affected eye in case of bilateral disease) was included in the study. The fellow eye was treated with the best treatment available in the center according to their clinical practice. During the study 043/SI, 13 out of 135 patients (9.6%) had bilateral AK. Nine study eyes of these patients with bilateral disease were treated with 0.8 mg/ml polihexanide and 4 with the combination of 0.2 mg/ml polihexanide and 1.0 mg/ml propamidine. The applicant did not clarify what therapy was actually implemented in the fellow-eye as best treatment according to clinical practice in patients with bilateral disease. No data on treatment and outcome of fellow eyes are available, thus no comparisons of the treated study eye and the fellow eye in subjects with bilateral disease are possible. Furthermore, no measures were in place to prevent treatment mistakes in case of bilateral disease with two distinct treatment therapies. Whether unnoticed treatment mistakes occurred in the 9.6% of subjects with bilateral disease that had to apply two distinct treatment therapies remains unclear. Importantly, no subject developed a bilateral infection during the study, all cases of bilateral disease were present from baseline. From reported data the bilateral infection did also not appear to crucially affect the resolution of the study eye and the exclusion of subjects with bilateral disease did not change the overall conclusion of the primary analysis.

Methodology

The clinical resolution rate (CRR) at 12 months from randomization, based on the percentage cured (intact corneal epithelium and uninflamed eye) defined by a set of clinical criteria (Oxford scale + inflammatory signs + scleritis as defined in the Protocol) at one month after stopping all therapy within 12 months of randomization, is the primary efficacy variable. As mild conjunctival inflammation was still allowed for AK to be considered resolved, the Applicant clarified that 5 subjects in the group treated with 0.8 mg/ml polihexanide and 2 in the group treated with 0.2 mg/ml polihexanide + 1.0 mg/ml propamidine had mild conjunctival inflammation while concluded as clinically resolved AK. Importantly, mild conjunctival inflammation (including bulbar injection, bulbar oedema, tarsal hyperaemia) was only acceptable if related to other concurrent conditions such as blepharitis. According to the study protocol, the presence of a mild conjunctival inflammation was allowed to consider AK resolved. For regulatory and verification reasons, an additional 60-day follow-up assessment of clinical resolution was applied to exclude late relapses. Analyses by additional time points before 1 year (e.g. 3 and 6 months) were requested during assessment. Data beyond 1 year should not be considered, as this would bias results in the direction of equivalence due to the treatment duration being maximal 12 months.

Secondary efficacy variables included time-to-cure (which is seen as key secondary), BCVA, pupil test (swinging light test), corneal scarring, ulceration severity, anterior chamber inflammation as well as EQ-5D and VFQ25 questionnaires. The primary and secondary outcomes reflect the clinical improvement as intended by therapy and appear acceptable.

Sample size calculation could be followed with respect to the initially intended superiority testing. The power is below 80% when testing for non-inferiority with a non-inferiority margin of 20% and a total

sample size of 130. As the test for non-inferiority is significant as applied (see critical discussion above and below) and the final decision for authorisation is mainly based on the comparison to external control data, the lower power is irrelevant for the interpretation of results. Randomisation was 1:1 and principally acceptable, however the lack of stratification factors is critically noted, especially with respect to identified imbalances (e.g. prior steroid use). Additional analyses adjusting for important prognostic factors were therefore requested and provided.

There is a small risk of disclosing of treatment assignment for subjects continuing to participate in the study because unmasking is allowed per protocol in case of lack of efficacy. As bottles could be distinguished, the masking of participants as well as of doctors carrying out the study could have been compromised. The decision as to which protocol deviations should be considered as reason for exclusion from the PPAS were made at the clean file meeting and documented in the clean file report. The clean file meeting occurred on July 1st 2021 after the last patient had had the last visit on 18 June 2021 and it was performed with blinded data.

Efficacy data and additional analyses

In total, 69 and 66 subjects were assigned to treatment with 0.8 mg/ml polihexanide + placebo and 0.2 mg/ml polihexanide + 1 mg/ml propamidine, respectively. All except one subject received at least one dose of study drug, resulting in 134 subjects in the Safety Analysis Set. The Full Analysis Set consists of 127 subjects (n=66 and n=61 for the monotherapy and the combination therapy, respectively) after 7 randomised subjects were excluded based on diagnosis criteria. Protocol compliance and required adjunctive therapy was comparable across both treatment groups (see a critical discussion on the subgroup of subjects with adjunctive therapy below). In total 8 major protocol deviations were recorded during study 043/SI. Protocol deviations were balanced across both treatment arms (n=4 per arm) and do not give rise to concern for the Per Protocol Analysis Set (n=119). Study treatment discontinuations (13.5% and 11.5% for the monotherapy and combination therapy, respectively) and study withdrawals (15.2% and 11.5% for the monotherapy and combination therapy, respectively) do not give rise to concern as they were balanced across treatment arms and mostly related to adverse events. One protocol amendment and one national GCP inspection at a study site in Poland did not give rise to major concerns regarding study conduct. Demographics were principally balanced, but baseline characteristics show imbalances with respect to prior medication (especially ocular steroids were used in around twice as many subjects in the monotherapy group, 47.8%, compared to subjects that received the combination therapy, 24.6%), some imbalances in medical history of eye disorders and reported eye pain as concurrent disorder (see discussion on safety aspects).

Regarding the primary outcome of trial 043, the 12-month clinical resolution rate is slightly lower for polihexanide +placebo (84.8% for FAS and 87.1% for the PPAS) compared to polihexanide +propamidine (88.5% for FAS and 88.5% for the PPAS). Thus, superiority was not demonstrated, as the difference is not statistically significant (neither at 5% nor at 10% significance level in either analysis set). The difference in proportion of clinical resolution rate is -0.04 with 90% CI (-0.14, 0.07) for the FAS and -0.02 with 90% CI (-0.13, 0.08) for the PPAS. The lower limit of the 90% CI is above the pre-defined non-inferiority margin of 20% in both analysis sets, i.e. polihexanide +placebo is not more than 20% worse than polihexanide +propamidine. Considering point estimates, the polihexanide +placebo group seems to perform worse than the polihexanide +propamidine group, but the 90% CI of the difference in 12-month clinical resolution rate still lies within the pre-defined but unjustified margin of 20%. Of note, the medical cure rate in the reference arm substantially differs from the assumptions made for the derivation of the non-inferiority margin (63% versus 88.5% (ITT)) which violates the constancy assumption for the margin derivation. A type I error rate of 10% was agreed to only for the primary superiority analysis. Thus, the lower bound of the 95% CI was requested. The 95% CI for the difference in proportion of clinical resolution rate was (-0.16, 0.09) in the FAS and (-0.15, 0.10) in the

PPASPPS. Upon request, the primary analysis was also adjusted for age, sex, prior exposure to steroids and staging of AK at baseline. After adjustment for these factors, the difference in proportion of resolution rate was -0.05 with a 95% CI of (-0.18, 0.09). Discontinued subjects are regarded as not cured in the primary analysis. Multiple imputation for missing data (patients withdrawn/discontinued due to a reason other than treatment failure) was performed under MAR accounting for treatment group and the difference in proportion resolved was -0.02 with 95% CI of (-0.12, 0.08). A tipping point analysis for missing data showed that all combinations except the worst case scenario generated a result with the lower 95% CI limit above -0.2. However, a difference of 0.2 (or the actually observed lower bound of the 95% CI of -0.16 in the FAS) in clinical resolution rate at 12 months cannot be called clinically irrelevant, and therefore Akantior cannot be called non-inferior to the active comparator as applied in the 043/SI trial.

Secondary endpoints largely appear similar between groups. The effects compared to baseline measured within each arm, although biased (e.g. bias due to variability in disease history, assessment bias due to knowledge of treatment, temporal variations of disease, regression to the mean), are mostly statistically significant. A presented Kaplan-Meier plot for time-to-cure shows that the proportion with clinical resolution after treatment with polihexanide +propamidine is consistently above the proportion of clinical resolution in polihexanide +placebo. Furthermore, the CRR at all presented time points before 12 months (i.e. 1, 2, 3, 4, 5 and 6 months) was lower for 0.8 mg/mL polihexanide than for the comparator. The differences between point estimates for all presented time-points ranged between approximately 3 and 16 percentage points. For the secondary endpoint "time to cure", a log-rank test for the difference between treatment groups was statistically significant in favour of the control arm (p=0.0442). The median time to cure was 140 days (117,150) for 0.8 mg/ml polihexanide and 114 days (91,127) for the active comparator arm. The group difference in change in BCVA was 0.05 with 95% CI (-0.08, 0.18) in favour of the active comparator arm. Still, the interpretation of the non-significant secondary endpoints is hampered by the fact that there is no non-inferiority margin defined for these endpoints and a non-significant p-value cannot be interpreted as there being no difference. Of note, the trial was not powered for showing an effect in these secondary endpoints.

Importantly, no relapse was documented during the 90-day study follow-up period off therapy after end-of-treatment. However, in four cases patients required an increase of the dose due to worsening of the condition, all of them in the monotherapy treatment group. There seems to have been no treatment switching or additional treatment for these patients. All these patients were counted as treatment failure in the primary analysis. There were small differences in the proportion of treatment failures (prematurely withdrawn subjects) between treatment groups of the combination treatment group (for the FAS 7/61, 11.5% and for the PPS 6/57, 10.5%) versus polihexanide 0.8 mg/ml (for the FAS 10/66, 15.2% and for the PPS 8/62 12.9%). Overall, 2 subjects experienced the event of corneal transplantation, both in the 0.8 mg/ml polihexanide + placebo treatment group (1 was coded as "Corneal infiltrates" and therefore, it was not included in the respective table as "Corneal transplant").

Forest plots for all relevant subgroups defined by risk factors (staging of *Acanthamoeba* keratitis at baseline, prior steroid treatment, region, age and sex) were provided for the difference in proportion of CRR after 12 months (CRR_12) and for the Odds Ratio. Subgroup analyses indicate that the subgroup with prior steroid use had a better resolution rate when treated with the combination treatment (CRR_12 was 77.4% in the polihexanide +placebo group and 90.0% in the polihexanide +propamidine group). This finding is of relevance, considering that twice as many patients were treated with prior steroids in the monotherapy group compared to the combination therapy group (47.8% and 24.6%, respectively). Deviations in treatment success based on prior corticosteroid use are also reported in literature (see Scruggs et al. 2022). However, a possible negative effect of prior steroid use became only apparent for the group treated with the monotherapy. Furthermore, most subjects with worsening of disease condition during treatment had reported prior steroid use (3 out of 4, all in the monotherapy

arm). It is unclear why no stratification for prior steroid use was implemented as apparently the effect appears obvious to the Applicant as stated in response to a requested clarification. However, also upon adjustment for important prognostic factors including prior exposure to steroids (besides age, sex and staging of AK at baseline; see details above) the difference in favour of the active control group even gets larger. Topical steroids as adjunctive therapy were used by 43 subjects (63.7%) in the monotherapy arm, whereas in the combination treatment arm only 33 subjects (54.1%) used topical steroids. A slightly higher percentage in the combination treatment arm started topical steroids during the trial, but this is probably due to a higher percentage starting before and continuing taking topical steroids in the monotherapy arm.

No differences within the subgroups with and without adjunctive treatments (i.e. topical steroids or oral NSAIDs added or increased after baseline) between treatment groups were observed, but failure in clinical resolution at 12 months is reported almost exclusively in subjects with adjunctive therapy for both groups (only in one subject without adjunctive therapy AK did not resolve). Subjects with adjunctive therapy were possibly at a more severe disease stage, which might have caused or contributed to worse outcomes in these patients. As steroids are mainly given with advanced AK staging and disease severity was evaluated only at baseline, it is unclear if the advanced stage of the disease or the concomitant treatment are the reason of observed differences.

Subgroup analysis considering staging of AK at baseline indicates better outcomes in CRR_12 following the applied combination treatment when diagnosed with stage I (85.7% for monotherapy and 100% for combination therapy) or stage III (63.6%% for monotherapy and 85.7% for combination therapy) AK. Stage II AK at baseline does not show differences to the same extent. The majority of subjects had stage II AK at baseline, but for the monotherapy more patients with stage III and stage I were included compared to more subjects with stage II in the combination therapy.

The systematic literature review (SLR) concluded a clinical resolution rate without surgery of 19.6% (95%CI:10.2%, 32.4%) for "untreated" patients that is based on identified observational studies and case reports. The remaining 80.4% of patients required surgery (keratoplasty 38/56 (0.68 [0.48, 0.83]), enucleation 4/56 (0.07 [0.03, 0.18]), minor surgery 4/56 (0.07 [0.01, 0.29])). Subjects requiring urgent surgical intervention for advanced *Acanthamoeba* keratitis in either eye (e.g., for advanced corneal thinning/melting etc.) were excluded from study 043. A comparison of population characteristics between the SLR and study 043 was provided concerning age and sex. Both characteristics appear principally comparable, even though more male patients were included in SLR of "untreated" patients (50% vs. 41.7%). Male subjects had a slightly worse resolution rate compared to women in study 043 (81.5% of males and 87.2% of female subjects with clinical resolution at 12 months). However, it is unclear whether a similar effect could be assumed for spontaneous resolution, which could influence the reported outcome. Of note, disease stage was not reported in any of the studies of "untreated" AK cases. Thus, disease stage could be an influential variable on reported resolution rates, as patients requiring urgent surgical intervention for AK were excluded from trial 043. Patients with advanced disease stage are considered less likely to resolve without treatment and thus could have influenced the results reported in the SLR. Importantly, patients with urgent need of ocular surgery due to advanced Acanthamoeba keratitis are contraindicated for the treatment with Akantior and resolution rates were favourable for Akantior also when considering a potential bias ("study effect") of 30.7% (see below).

For the estimation of efficacy of the active control (combination) treatment applied in study 043, a systematic literature review of the outcomes for all treatments for *Acanthamoeba* keratitis was conducted in 2022. The cure definition used in the pivotal trial 043/SI was claimed to be fully comparable only with the expanded retrospective study published by Papa et al. 2020. Of note, the cure rate of the polihexanide monotherapy was identified to be the lowest for all compared anti-amoebic treatments but was applied in a lower concentration compared to study 043. In the original

SIFI /Moorfields observational study (i.e. before the publication of Papa et al. 2020), the expected clinical resolution rate in the group treated with the combination therapy (0.2 mg/ml polihexanide plus 1 mg/ml propamidine) was 67%, which was lowered to 63% due to assumed poorer outcomes in the patients with advanced disease. However, the expanded retrospective study published by Papa et al. 2020 concluded a cure rate of 57.8% and cure rates observed during trial 043 were 88.5%. The outcomes in the trial are substantially higher than observed for cure rates in real-world settings and, therefore, any indirect comparison seems questionable as the constancy assumption is violated. The applicant assumes that Akantior will have a similar effect in the real world setting as in the pivotal study. The claim that observed differences are due to medicinal product manufactured to GMP quality, immediate availability and a pragmatic, easy-to-use, standardized, clear treatment protocol was considered speculative. A so-called "study effect" of 30.7% (95%CI: 14.2%; 47.2%) was concluded by the Applicant comparing results observed for the chosen comparator in study 043 and Papa et al. 2020. When performing a crude adjustment method of adding this potential bias of 30.7% between observational data and study 043 to the results of the SLR of "untreated" patients, the estimated resolution rate of "untreated" patients in a study setup as applied in study 043 is 50.3% (95%CI: 36.6%; 64.1%).

Of note, 95% CIs do not overlap between the primary endpoint measure from trial 043 (84.8% with 95%CI: 73.9%; 92.5%) and the resolution rates estimated from historical "untreated" controls (as per systematic literature review: 19.6% (95%CI: 10.2%, 32.4%)) even when adding this study effect of 30.7% (50.3% with 95%CI: 36.6%, 64.1%). This indicates that there is a significant difference in proportions. Upon request, the Applicant provided the 95% CI for the difference in proportions in CRR at 12 months for the comparison of polihexanide 0.8 mg/ml with "untreated" control cases with and without adding the estimated study effect. The difference in proportions in CRR at 12 months between Akantior from study 043 and "untreated" cases from the SLR without subtracting a study effect is 65.2% (with 95% CI: 49.3%, 77.5%) and subtracting a study effect of 30.7% is 34.5% (with 95% CI: 16.8%, 49.8%). Considering that the study endpoint is rather of objective nature (resolution from infection) and the outcome on treatment is markedly different from that of the external control, a benefit over no treatment can be concluded for Akantior (see also ICH E10). Also, a mixed treatment comparison (MTC) synthesis, which is also known as network meta-analysis was applied. The results of the MTC with and without bias-adjustment showed that polihexanide 0.8 mg/ml remained associated with statistically significant higher clinical resolution rate than no treatment and are similar to results using the crude adjustment method.

In summary, the effect of Akantior observed in study 043 appears strong and Akantior performs better than historical control data even when considering a possible large bias between data sets. External validity of the magnitude of treatment effect is currently not fully certain, considering that an external control group had to be used and the fact that the intended commercial DP could not be assessed critically after clinical use. Still, based on available data it can be concluded that Akantior performs better than no antiamoebic treatment also in clinical practice.

2.6.7. Conclusions on the clinical efficacy

Superiority against the chosen comparator comparator was not shown in the pivotal study and methodological uncertainties remain regarding non-inferiority against the comparator utilised in study 043. Therefore, Akantior cannot be called non-inferior to the comparator.

As it is unrealistic to expect that a test treatment could be superior to unapproved SOC with comparable/same mechanism of action in a well-managed CT setting and it is unethical to randomise to placebo, the only option was an indirect comparison to "untreated" AK patients. The treatment effect of interest is defined as the comparison (contrast) of the summary measure under the

experimental treatment to the summary measure under the alternative of the trial population not being treated with the experimental treatment (counterfactual). The absolute benefit of Akantior compared to no antiamoebic treatment has been shown when comparing the active treatment arm to data from literature, i.e. considering the pivotal trial rather a SAT than a RCT and relying on external control data. Akantior performs better than historical control data even when considering a possible large bias between data sets and the effect of Akantior observed in study 043 is strong. External validity of the magnitude of treatment effect is uncertain, but based on available data it can be concluded that Akantior performs better than no antiamoebic treatment also in clinical practice. Thus, Akantior is considered an efficacious therapy for the treatment of *Acanthamoeba* keratitis.

The use of DS from Supplier Y for the commercial DP (instead of the DS from Supplier X employed for clinical studies) does not appear to crucially impact the expected efficacy and safety profile of Akantior.

2.6.8. Clinical safety

The safety evaluation of 0.8 mg/mL polihexanide was based on the Phase I study 042/SI including 27 healthy volunteers and the Phase III study 043/SI including 69 patients with *Acanthamoeba* keratitis.

Study 042/SI

The study evaluated the ocular safety and tolerability and the systemic safety of 3 different doses of preservative-free polihexanide (0.4 mg/mL, 0.6 mg/mL and 0.8 mg/mL) compared to placebo in healthy subjects. The study was designed as randomised, double-masked, placebo-controlled, multiple centres, parallel group Phase I study. The purpose of the study was to determine whether any treatment group was at significantly greater risk of having an AE than placebo and to select the highest tolerated dose of polihexanide to be included as treatment arm in the pivotal clinical trial. Ninety subjects were assigned to one of the four treatment groups at a ratio of 3:3:3:1 and dosed with 1 drop every hour (12 administrations a day) for 1 week and 1 drop every 2 hours (6 administrations a day) for an additional week. The safety and tolerability assessments during the study comprised collection of Adverse Events, clinical laboratory (haematology, biochemistry, and urinalysis), vital signs and ocular safety parameters (best corrected visual acuity, slit-lamp examination, ocular surface fluorescein and lissamine green staining, ophthalmoscopy, intraocular pressure, assessment of ocular discomfort and global subjective tolerance (ocular surface disease index [OSDI]).

Population for the safety analysis: Safety data for all subjects who have received at least 1 dose of study medication was analyzed in the safety analyses.

Study 043/SI

The safety profile of 0.8 mg/mL polihexanide is based primarily on the pivotal study 043/SI. The study was designed as a randomised, assessor-masked, double-dummy, active-controlled, multicentre, parallel-group Phase III study to evaluate the efficacy, safety, and tolerability of 0.8 mg/mL polihexanide ophthalmic solution, as monotherapy, compared to the treatment combination of 0.2 mg/mL polihexanide + 1.0 mg/mL propamidine in adults and adolescent subjects (aged >12 years) affected by *Acanthamoeba* keratitis. The study protocol excluded subjects treated with drugs having effects on *Acanthamoeba* prior to study entry and subjects with concomitant viral or fungal infections or requiring systemic immunosuppression. In the study 043/SI a total of 134 patients (Safety subset) received at least 1 study treatment: 69 patients were exposed to 0.8 mg/mL polihexanide + placebo (treatment group) and 65 patients were exposed to 0.2 mg/mL polihexanide plus 1.0 mg/mL propamidine (control group) for up to 12 months. The study consisted of an eligibility screening visit, a treatment period including ambulant visits, and 2 off-therapy follow-up visits (30-days and 90-days after the end of treatment). The maximum treatment period allowed was 1 year. Safety and tolerability

evaluations comprised AE reporting, clinical laboratory (haematology, biochemistry and urinalysis), IOP, ophthalmoscopy, and photography of affected cornea.

2.6.8.1. Patient exposure

Overall exposure to 0.8 mg/mL polihexanide included 27 healthy volunteers who had a planned exposure of 2 weeks in Study 042/SI and 69 patients with *Acanthamoeba* keratitis who were exposed for approximately 35 weeks (max. up to one year) in Study 043/SI. The mean drug exposure of patients with *Acanthamoeba* keratitis to Akantior was 138.3 ± 79.6 days.

Table 66. Extent of exposure, Study 042

	PHMB				
	0.04% (N=26)	0.06% (N=28)	0.08% (N=27)	Placebo (N=9)	Total (N=90)
Patients, whose drug was dispensed per protocol					
Visit 2	26 (100%)	28 (100%)	27 (100%)	9 (100%)	90 (100%)
Visit 3	26 (100%)	26 (92.9%)	23 (85.2%)	9 (100%)	84 (93.3%)
Number of unused vials collected, mean (SD)[a]					
Visit 3	13.1 (3.2)	12.2 (2.1)	12.5 (2.0)	11.7 (2.8)	12.5 (2.6)
Visit 4	8.4 (2.1)	9.0 (1.8)	8.6 (0.9)	8.2 (1.1)	8.6 (1.7)
Visit 5 (follow-up)	NA (NA)	56.5 (24.7)	61.5 (17.9)	NA (NA)	59.8 (17.9)

Percentages are based on number of subjects in the Safety analysis set.
SD = Standard deviation
[a] The means (SD) are based only on the number of subjects who did return unused vials at the visit.
This table is also presented in Section 14.2 (Table 10).

Table 67. Extent of exposure, Study 043

Duration (days)	0.8 mg/mL polihexanide + placebo (N=69)	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=65)	Total (N=134)
Total (all visits)			
n/nmiss	69/0	65/0	134/0
Mean (SD)	138.3 (79.6)	113.9 (52.5)	126.4 (68.7)
Median	120.0	100.0	115.5
Q1, Q3	92.0, 152.0	71.0, 152.0	89.0, 152.0
Min, Max	10, 387	28, 233	10, 387

Source: Study 043/SI Clinical Study Report (CSR) Table 14.3.1.1
If a patient has a missing end of treatment date, the end of study date will be used to calculate duration.
n/nmiss = number of subjects with evaluable/missing data; Q1 = first quartile; Q3 = third quartile;
SD = standard deviation.

In the pivotal study the median duration of exposure was longer in the 0.8 mg/ml polihexanide + placebo treatment group (120.0 days [Range: 10.0-387.0]) in comparison to the duration of exposure in the 0.2 mg/ml polihexanide + 1 mg/ml propamidine treatment group (100.0 days [Range: 28.0-233.0]).

2.6.8.2. Adverse events

Study 042/SI

Table 68. Frequency of adverse events in healthy volunteers (Study 042, SAS)

	Polihexanide									
	0.4 mg/mL (N=26)		0.6 mg/mL (N=28)		0.8 mg/mL (N=27)		Placebo (N=9)		Total (N=90)	
	n (%)	m	n (%)	m	n (%)	m	n (%)	m	n (%)	m
Any AE	11 (42.3)	19	23 (82.1)	67	16 (59.3)	48	5 (55.6)	11	55 (61.1)	145
Any TEAE	10 (38.5)	17	22 (78.6)	66	16 (59.3)	44	5 (55.6)	11	53 (58.9)	138
Any SAE	0	0	0	0	0	0	0	0	0	0
Any DLE	0	0	2 (7.1)	3	3 (11.1)	6	0	0	5 (5.6)	9
Any AE leading to withdrawal	0	0	2 (7.1)	2	3 (11.1)	6	0	0	5 (5.6)	8
Any AE leading to death	0	0	0	0	0	0	0	0	0	0
AEs by relationship										
Unrelated	3 (11.5)	3	9 (32.1)	9	7 (25.9)	11	4 (44.4)	5	23 (25.6)	28
Unlikely	2 (7.7)	2	4 (14.3)	5	0	0	1 (11.1)	2	7 (7.8)	9
Possible	1 (3.8)	3	2 (7.1)	4	1 (3.7)	1	0	0	4 (4.4)	8
Probable	4 (15.4)	6	9 (32.1)	22	8 (29.6)	21	0	0	21 (23.3)	49
Related	3 (11.5)	5	5 (17.9)	27	6 (22.2)	15	2(22.2)	4	16 (17.8)	51
AEs by intensity[1]										
Mild	10 (38.5)	18	20 (71.4)	55	16 (59.3)	41	5(55.6)	11	51 (56.7)	125
Moderate	1 (3.8)	1	9 (32.1)	12	3 (11.1)	7	0	0	13 (14.4)	20
Severe	0	0	0	0	0	0	0	0	0	0
Life-threatening	0	0	0	0	0	0	0	0	0	0
Leading to death	0	0	0	0	0	0	0	0	0	0

Source: CSR 042/SI Section 14, Table 12

AE = adverse event; DLE = dose limiting event; m = number of mentions; n = number of subjects; SAE = serious adverse event; TEAE = treatment-emergent adverse event.

Percentages are based on number of subjects in the Safety analysis set.

Table 69. Frequency of treatment-emergent adverse events in healthy volunteers (Study 042, SAS)

	Polihexanide 0.4 mg/mL (N=26)		Polihexanide 0.6 mg/mL (N=28)		Polihexanide 0.8 mg/mL (N=27)		Placebo (N=9)		Total (N=90)	
System organ class Preferred term	n (%)	m	n (%)	m	n (%)	m	n (%)	m	n (%)	m
Any TEAE	10 (38.5)	17	22 (78.6)	66	16 (59.3)	44	5 (55.6)	11	53 (58.9)	138
Blood and lymphatic system disorders	0	0	0	0	0	0	1 (11.1)	1	1 (1.1)	1
Anaemia	0	0	0	0	0	0	1 (11.1)	1	1 (1.1)	1
Eye disorders	4 (15.4)	5	13 (46.4)	27	9(33.3)	10	1 (11.1)	4	27 (30.0)	46
Abnormal sensation in eye	0	0	0	0	0	0	1 (11.1)	2	1 (1.1)	2
Conjunctival follicles	2 (7.7)	2	0	0	0	0	0	0	2 (2.2)	2
Conjunctival hyperaemia	1 (3.8)	1	4 (14.3)	4	4 (14.8)	4	0	0	9 (10.0)	9
Conjunctival irritation	0	0	3 (10.7)	3	1 (3.7)	1	0	0	4 (4.4)	4
Conjunctival oedema	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
Conjunctivitis allergic	0	0	1 (3.6)	1	2 (7.4)	2	0	0	3 (3.3)	3
Erythema of eyelid	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
Eye discharge	1 (3.8)	1	3 (10.7)	3	0	0	0	0	4 (4.4)	4
Eye irritation	0	0	0	0	0	0	1 (11.1)	1	1 (1.1)	1
Eye pain	0	0	1 (3.6)	1	0	0	1 (11.1)	1	2 (2.2)	2
Eyelid cyst	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
Eyelid oedema	0	0	6 (21.4)	6	0	0	0	0	6 (6.7)	6
Lacrimation increased	0	0	0	0	1 (3.7)	1	0	0	1 (1.1)	1
Ocular discomfort	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
Ocular hyperaemia	1 (3.8)	1	1 (3.6)	1	0	0	0	0	2 (2.2)	2
Photophobia	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
Retinal pigment epitheliopathy	0	0	0	0	1 (3.7)	1	0	0	1 (1.1)	1
Vision blurred	0	0	2 (7.1)	3	1 (3.7)	1	0	0	3 (3.3)	4
Gastrointestinal disorders	0	0	0	0	2 (7.4)	2	0	0	2 (2.2)	2
Dental caries	0	0	0	0	1 (3.7)	1	0	0	1 (1.1)	1
Vomiting	0	0	0	0	1 (3.7)	1	0	0	1 (1.1)	1
General disorders and administration site conditions	4 (15.4)	6	7 (25.0)	15	6 (22.2)	9	2 (22.2)	2	19 (21.1)	32
Instillation site dryness	0	0	1 (3.6)	1	2 (7.4)	2	0	0	3 (3.3)	3
Instillation site erythema	2 (7.7)	2	3 (10.7)	4	0	0	0	0	5 (5.6)	6
Instillation site foreign body sensation	1 (3.8)	1	2 (7.1)	2	1 (3.7)	1	1 (11.1)	1	5 (5.6)	5
Instillation site lacrimation	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
Instillation site pain	2 (7.7)	2	4 (14.3)	4	5 (18.5)	5	1 (11.1)	1	12 (13.3)	12
Instillation site pruritus	1 (3.8)	1	3 (10.7)	3	1 (3.7)	1	0	0	5 (5.6)	5
Infections and infestations	0	0	7 (25.0)	7	1 (3.7)	1	2 (22.2)	2	10 (11.1)	10
Conjunctivitis	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
Influenza	0	0	1 (3.6)	1	1 (3.7)	1	1 (11.1)	1	3 (3.3)	3
Nasopharyngitis	0	0	3 (10.7)	3	0	0	1 (11.1)	1	4 (4.4)	4
Oral herpes	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1

Urinary tract infection	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
Injury, poisoning and procedural complications	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
Contusion	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
Investigations	2 (7.7)	3	6 (21.4)	10	11 (40.7)	19	0	0	19 (21.1)	32
Conjunctival staining	2 (7.7)	2	5 (17.9)	5	9 (33.3)	11	0	0	16 (17.8)	18
Vital dye staining cornea	1 (3.8)	1	5 (17.9)	5	8 (29.6)	8	0	0	14 (15.6)	14
Musculoskeletal and connective tissue disorders	0	0	0	0	1 (3.7)	1	1 (11.1)	1	2 (2.2)	2
Arthralgia	0	0	0	0	1 (3.7)	1	0	0	1 (1.1)	1
Pain in jaw	0	0	0	0	0	0	1 (11.1)	1	1 (1.1)	1
Nervous system disorders	3 (11.5)	3	4 (14.3)	5	1 (3.7)	1	1(11.1)	1	9 (10.0)	10
Headache	3 (11.5)	3	4 (14.3)	5	1 (3.7)	1	1(11.1)	1	9 (10.0)	10
Reproductive system and breast disorders	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
Dysmenorrhea	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
Respiratory, thoracic and mediastinal disorders	0	0	0	0			0	0	1 (1.1)	1
Epistaxis	0	0	0	0			0	0	1 (1.1)	1

Source: CSR 042/SI Section 14, Table 13

m = number of mentions; n = number of subjects; TEAE = treatment-emergent adverse event.
Adverse events were coded according to MedDRA 19.0

Table 70.

Adverse events by severity that occurred in healthy volunteers (Study 042, SAS)

		Polihexanide									
		0.4 mg/mL (N=26)		0.6 mg/mL (N=28)		0.8 mg/mL (N=27)		Placebo (N=9)		Total (N=90)	
System organ class	Intensity	n (%)	m	n (%)	m	n (%)	m	n (%)	m	n (%)	m
Any TEAE											
	Mild	9 (34.6)	16	19 (67.9)	54	16 (59.3)	37	5 (55.6)	11	49 (54.4)	118
	Moderate	1 (3.8)	1	9 (32.1)	12	3 (11.1)	7	0	0	13 (14.4)	20
	Severe	0	0	0	0	0	0	0	0	0	0
	Life-threatening	0	0	0	0	0	0	0	0	0	0
	Leading to death	0	0	0	0	0	0	0	0	0	0
Blood and lymphatic system disorders		0	0	0	0	0	0	1 (11.1)	1	1 (1.1)	1
	Mild	0	0	0	0	0	0	1 (11.1)	1	1 (1.1)	1
	Moderate	0	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0	0
	Life-threatening	0	0	0	0	0	0	0	0	0	0
	Leading to death	0	0	0	0	0	0	0	0	0	0
Eye disorders		4 (15.4)	5	13 (46.4)	27	9 (33.3)	10	1 (11.1)	4	27 (30.0)	46
	Mild	3 (11.5)	4	12 (42.9)	25	8 (29.6)	9	1 (11.1)	4	24 (26.7)	42
	Moderate	1 (3.8)	1	1 (3.6)	2	1 (3.7)	1	0	0	3 (3.3)	4
	Severe	0	0	0	0	0	0	0	0	0	0
	Life-threatening	0	0	0	0	0	0	0	0	0	0
	Leading to death	0	0	0	0	0	0	0	0	0	0

Gastrointestinal disorders		0	0	0	0	2 (7.4)	2	0	0	2 (2.2)	2
	Mild	0	0	0	0	2 (7.4)	2	0	0	2 (2.2)	2
	Moderate	0	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0	0
	Life-threatening	0	0	0	0	0	0	0	0	0	0
	Leading to death	0	0	0	0	0	0	0	0	0	0
General disorders and administration site conditions		4 (15.4)	6	7 (25.0)	15	6 (22.2)	9	2 (22.2)	2	19 (21.1)	32
	Mild	4 (15.4)	6	6 (21.4)	11	5 (18.5)	6	2 (22.2)	2	17 (18.9)	25
	Moderate	0	0	4 (14.3)	4	2 (7.4)	3	0	0	6 (6.7)	7
	Severe	0	0	0	0	0	0	0	0	0	0
	Life-threatening	0	0	0	0	0	0	0	0	0	0
	Leading to death	0	0	0	0	0	0	0	0	0	0
Infections and infestations		0	0	7 (25.0)	7	1 (3.7)	1	2 (22.2)	2	10 (11.1)	10
	Mild	0	0	7 (25.0)	7	1 (3.7)	1	2 (22.2)	2	10 (11.1)	10
	Moderate	0	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0	0
	Life-threatening	0	0	0	0	0	0	0	0	0	0
	Leading to death	0	0	0	0	0	0	0	0	0	0
Injury, poisoning and procedural complications		0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
	Mild	0	0	0	0	0	0	0	0	0	0
	Moderate	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
	Severe	0	0	0	0	0	0	0	0	0	0
	Life-threatening	0	0	0	0	0	0	0	0	0	0
	Leading to death	0	0	0	0	0	0	0	0	0	0
Investigations		2 (7.7)	3	6 (21.4)	10	11 (40.7)	19	0	0	19 (21.1)	32
	Mild	2 (7.7)	3	5 (17.9)	7	9 (33.3)	16	0	0	16 (17.8)	26
	Moderate	0	0	2 (7.1)	3	3 (11.1)	3	0	0	5 (5.6)	6
	Severe	0	0	0	0	0	0	0	0	0	0
	Life-threatening	0	0	0	0	0	0	0	0	0	0
	Leading to death	0	0	0	0	0	0	0	0	0	0
Musculoskeletal and connective tissue disorders		0	0	0	0	1 (3.7)	1	1 (11.1)	1	2 (2.2)	2
	Mild	0	0	0	0	1 (3.7)	1	1 (11.1)	1	2 (2.2)	2
	Moderate	0	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0	0
	Life-threatening	0	0	0	0	0	0	0	0	0	0
	Leading to death	0	0	0	0	0	0	0	0	0	0
Nervous system disorders		3 (11.5)	3	4 (14.3)	5	1 (3.7)	1	1 (11.1)	1	9 (10.0)	10
	Mild	3 (11.5)	3	3 (10.7)	4	1 (3.7)	1	1 (11.1)	1	8 (8.9)	9
	Moderate	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
	Severe	0	0	0	0	0	0	0	0	0	0
	Life-threatening	0	0	0	0	0	0	0	0	0	0
	Leading to death	0	0	0	0	0	0	0	0	0	0
Reproductive system and breast disorders		0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
	Mild	0	0	0	0	0	0	0	0	0	0
	Moderate	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
	Severe	0	0	0	0	0	0	0	0	0	0
	Life-threatening	0	0	0	0	0	0	0	0	0	0
	Leading to death	0	0	0	0	0	0	0	0	0	0
Respiratory, thoracic and mediastinal disorders		0	0	0	0	1 (3.7)	1	0	0	1 (1.1)	1
	Mild	0	0	0	0	1 (3.7)	1	0	0	1 (1.1)	1
	Moderate	0	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0	0
	Life-threatening	0	0	0	0	0	0	0	0	0	0
	Leading to death	0	0	0	0	0	0	0	0	0	0

Source: CSR 042/SI Section 14, Table 16
m = number of mentions; n = number of subjects.
Adverse events are coded according to MedDRA 19.0
Percentages are based on number of subjects in the Safety analysis set

Table 71. Adverse events by causality that occurred in healthy volunteers (Study 042, SAS)

System organ class	Relationship	Polihexanide 0.4 mg/mL (N=26) n (%)	m	Polihexanide 0.6 mg/mL (N=28) n (%)	m	Polihexanide 0.8 mg/mL (N=27) n (%)	m	Placebo (N=9) n (%)	m	Total (N=90) n (%)	m
Any TEAE											
	Unrelated	1 (3.8)	1	8 (28.6)	8	6 (22.2)	7	4 (44.4)	5	19 (21.1)	21
	Unlikely	2 (7.7)	2	4 (14.3)	5	0	0	1 (11.1)	2	7 (7.8)	9
	Possible	1 (3.8)	3	2 (7.1)	4	1 (3.7)	1	0	0	4 (4.4)	8
	Probable	4 (15.4)	6	9 (32.1)	22	8 (29.6)	21	0	0	21 (23.3)	49
	Related	3 (11.5)	5	5 (17.9)	27	6 (22.2)	15	2 (22.2)	4	16 (17.8)	51
Blood and lymphatic system disorders		0	0	0	0	0	0	1 (11.1)	1	1 (1.1)	1
	Unrelated	0	0	0	0	0	0	1 (11.1)	1	1 (1.1)	1
	Unlikely	0	0	0	0	0	0	0	0	0	0
	Possible	0	0	0	0	0	0	0	0	0	0
	Probable	0	0	0	0	0	0	0	0	0	0
	Related	0	0	0	0	0	0	0	0	0	0
Eye disorders		4 (15.4)	5	13 (46.4)	27	9 (33.3)	10	1 (11.1)	4	27 (30.0)	46
	Unrelated	0	0	0	0	1 (3.7)	1	0	0	1 (1.1)	1
	Unlikely	0	0	0	0	0	0	1 (11.1)	2	1 (1.1)	2
	Possible	1 (3.8)	2	2 (7.1)	4	1 (3.7)	1	0	0	4 (4.4)	7
	Probable	3 (11.5)	3	8 (28.6)	9	5 (18.5)	5	0	0	16 (17.8)	17
	Related	0	0	4 (14.3)	14	2 (7.4)	3	1 (11.1)	2	7 (7.8)	19
Gastrointestinal disorders		0	0	0	0	2 (7.4)	2	0	0	2 (2.2)	2
	Unrelated	0	0	0	0	2 (7.4)	2	0	0	2 (2.2)	2
	Unlikely	0	0	0	0	0	0	0	0	0	0
	Possible	0	0	0	0	0	0	0	0	0	0
	Probable	0	0	0	0	0	0	0	0	0	0
	Related	0	0	0	0	0	0	0	0	0	0
General disorders and administration site conditions		4 (15.4)	6	7 (25.0)	15	6 (22.2)	9	2 (22.2)	2	19 (21.1)	32
	Unrelated	0	0	0	0	0	0	0	0	0	0
	Unlikely	0	0	0	0	0	0	0	0	0	0
	Possible	1 (3.8)	1	0	0	0	0	0	0	1 (1.1)	1
	Probable	0	0	2 (7.1)	2	1 (3.7)	1	0	0	3 (3.3)	3
	Related	3 (11.5)	5	5 (17.9)	13	5 (18.5)	8	2 (22.2)	2	15 (16.7)	28
Infections and infestations		0	0	7 (25.0)	7	1 (3.7)	1	2 (22.2)	2	10 (11.1)	10
	Unrelated	0	0	6 (21.4)	6	1 (3.7)	1	2 (22.2)	2	9 (10.0)	9
	Unlikely	0	0	0	0	0	0	0	0	0	0
	Possible	0	0	0	0	0	0	0	0	0	0
	Probable	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
	Related	0	0	0	0	0	0	0	0	0	0
Injury, poisoning and procedural complications		0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
	Unrelated	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
	Unlikely	0	0	0	0	0	0	0	0	0	0
	Possible	0	0	0	0	0	0	0	0	0	0
	Probable	0	0	0	0	0	0	0	0	0	0
	Related	0	0	0	0	0	0	0	0	0	0
Investigations		2 (7.7)	3	6 (21.4)	10	11 (40.7)	19	0	0	19 (21.1)	32
	Unrelated	0	0	0	0	0	0	0	0	0	0
	Unlikely	0	0	0	0	0	0	0	0	0	0
	Possible	0	0	0	0	0	0	0	0	0	0
	Probable	2 (7.7)	3	6 (21.4)	10	8 (29.6)	15	0	0	16 (17.8)	28
	Related	0	0	0	0	3 (11.1)	4	0	0	3 (3.3)	4

Musculoskeletal and connective tissue disorders		0	0	0	0	1 (3.7)	1	1 (11.1)	1	2 (2.2)	2
	Unrelated	0	0	0	0	1 (3.7)	1	1 (11.1)	1	2 (2.2)	2
	Unlikely	0	0	0	0	0	0	0	0	0	0
	Possible	0	0	0	0	0	0	0	0	0	0
	Probable	0	0	0	0	0	0	0	0	0	0
	Related	0	0	0	0	0	0	0	0	0	0
Nervous system disorders		3 (11.5)	3	4 (14.3)	5	1 (3.7)	1	1 (11.1)	1	9 (10.0)	10
	Unrelated	1 (3.8)	1	0	0	1 (3.7)	1	1 (11.1)	1	3 (3.3)	3
	Unlikely	2 (7.7)	2	4 (14.3)	5	0	0	0	0	6 (6.7)	7
	Possible	0	0	0	0	0	0	0	0	0	0
	Probable	0	0	0	0	0	0	0	0	0	0
	Related	0	0	0	0	0	0	0	0	0	0
Reproductive system and breast disorders		0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
	Unrelated	0	0	1 (3.6)	1	0	0	0	0	1 (1.1)	1
	Unlikely	0	0	0	0	0	0	0	0	0	0
	Possible	0	0	0	0	0	0	0	0	0	0
	Probable	0	0	0	0	0	0	0	0	0	0
	Related	0	0	0	0	0	0	0	0	0	0
Respiratory, thoracic and mediastinal disorders		0	0	0	0	1 (3.7)	1	0	0	1 (1.1)	1
	Unrelated	0	0	0	0	1 (3.7)	1	0	0	1 (1.1)	1
	Unlikely	0	0	0	0	0	0	0	0	0	0
	Possible	0	0	0	0	0	0	0	0	0	0
	Probable	0	0	0	0	0	0	0	0	0	0
	Related	0	0	0	0	0	0	0	0	0	0

Source: CSR 042/SI Section 14, Table 15
m = number of mentions; n = number of subjects.
Adverse events are coded according to MedDRA 19.0
Percentages are based on number of subjects in the Safety analysis set

Table 72. Most common (> 5%) TEAEs in healthy volunteers (Study 042)

Preferred Term (PT)	Polihexanide 0.4 mg/mL (n=26)		Polihexanide 0.6 mg/mL (n=28)		Polihexanide 0.8 mg/mL (n=27)		Placebo (n=9)		Total (n=90)	
	N (%)	m	N (%)	m	N (%)	m	N (%)	m	N (%)	m
Conjunctival staining	2 (7.7)	2	5 (17.9)	5	9 (33.3)	11	0	0	16 (17.8)	18
Corneal staining	1 (3.8)	1	5 (17.9)	5	8 (29.6)	8	0	0	14 (15.6)	14
Instillation site pain	2 (7.7)	2	4 (14.3)	4	5 (18.5)	5	1 (11.1)	1	12 (13.3)	12
Headache	3 (11.5)	3	4 (14.3)	5	1 (3.7)	1	1 (11.1)	1	9 (10.0)	10
Conjunctival hyperemia	1 (3.8)	1	4 (14.3)	4	4 (14.8)	4	0	0	9 (10.0)	9
Eyelid edema	0	0	6 (21.4)	6	0	0	0	0	6 (6.7)	6
Instillation site erythema	2 (7.7)	2	3 (10.7)	4	0	0	0	0	5 (5.6)	6
Instillation site foreign body sensation	1 (3.8)	1	2 (7.1)	2	1 (3.7)	1	1 (11.1)	1	5 (5.6)	5
Instillation site pruritus	1 (3.8)	1	3 (10.7)	3	1 (3.7)	1	0	0	5 (5.6)	5

Source: Study 042/SI CSR, Table 13, Section 14.2.1.

Ocular safety assessments: Statistically significant treatment differences between dose groups and placebo were observed with regards to assessment of BCVA, slit-lamp examination, vital staining of the ocular surface (cornea) and in self-assessments of ocular discomfort and OSDI scores. No statistically

significant differences between dose groups and placebo were observed for the slit-lamp examination, ophthalmoscopy assessments and IOP.

- BCVA: in the left eye (clean control) there was a statistically significant decrease in visual acuity in the left eye in all dose groups compared to placebo but the treatment differences were small and of no clinical significance.
- Slit lamp examination: A statistically significant difference between the 0.06% polihexanide group and the placebo group with regard to the conjunctiva erythema grade in the right eye (treated) was observed at Visit 3 (OR: 0.040 [95% CI= 0.002 to 0.636]; p=0.0224) showing a considerably lower probability in the 0.06% polihexanide group for the outcome "No conjunctiva erythema" compared to the placebo group.
- Vital Staining of the Ocular Surface (cornea and conjunctiva): In the right eye (treated), there were statistically significant increases in vital staining of the cornea (fluorescein) in all dose groups compared to placebo at Visit 3/Day 7. At Visit 4/Day 14, there was a statistically significant increase in vital staining of the cornea in the 0.08% dose group compared to placebo. In the left eye (clean control), there was a statistically significant increase in vital staining of the cornea in the 0.04% polihexanide group compared to placebo at Visit 3/Day 7.
- Ocular Discomfort (VAS scale): In the right eye (treated), the difference in adjusted mean change from baseline compared to placebo in ocular discomfort was 11.78 mm (95% CI= 2.88 to 20.69; p=0.0101) in the 0.06% polihexanide group and 14.87 mm in the 0.08% polihexanide group at Visit 3/Day 7. At Visit 4/Day 14, a statistically significant increase in ocular discomfort in the right eye was only observed in the 0.08% polihexanide dose group (treatment difference: 8.81 mm [95% CI= 2.53 to 15.08; p=0.0065]) whereas at Visit 5/Day 21 (follow-up), a statistically significant increase in ocular discomfort was only observed in the 0.06% polihexanide dose group. In the left eye (clean control), At Visit 4/Day 14, a small but statistically significant increase in ocular discomfort in the left eye was observed in all dose groups compared to placebo (the treatment differences were 1.99 mm. At Visit 5/Day 21 (follow-up), a small but statistically significant increase in ocular discomfort in the right eye was only observed in the 0.08% polihexanide dose group compared to placebo.
- OSDI scores: mean OSDI scores increased in all dose groups except placebo between baseline and Visits 3 and 4 (Days 7 and 14) which indicates a worsening of the visual functioning. A statistically significant increase in OSDI scores compared to placebo was observed in the 0.06% polihexanide and 0.08% polihexanide groups at Visit 3/Day 7, in the 0.08% polihexanide group at Visit 4 (Day 14) and in the 0.06% polihexanide group at Visit 5/Day 21 (follow-up). In conclusion, the treatment difference compared to placebo in mean change from baseline in OSDI scores decreased over time in all dose groups and were more or less normalised at the follow-up visit on Day 21

Study 043/SI

Table 73. Overview of adverse events in patients with *Acanthamoeba* keratitis (Study 043, SAS)

	0.8 mg/mL polihexanide + placebo (N=69)		0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=65)		Total (N=134)	
	n (%)	m	n (%)	m	n (%)	m
Any adverse event	31 (44.9)	83	29 (44.6)	69	60 (44.8)	152
Any serious adverse event	2 (2.9)	3	1 (1.5)	1	3 (2.2)	3
Any adverse event leading to death	0	0	0	0	0	0
Adverse events by toxicity						
Severity grade I-II (Mild, Moderate)	29 (42.0)	76	28 (43.1)	63	57 (42.5)	139
Severity grade III-V (Severe, Life-threatening, Death)	4 (5.8)	7	5 (7.7)	6	9 (6.7)	13
Adverse event by action taken with study treatment						
Dose increased	5 (7.2)	7	0	0	5 (3.7)	7
Dose not changed	24 (34.8)	48	23 (35.4)	57	47 (35.1)	105
Dose reduced	0	0	0	0	0	0
Drug interrupted	11 (15.9)	16	6 (9.2)	6	17 (12.7)	22
Not applicable	6 (8.7)	12	5 (7.7)	6	11 (8.2)	18
Unknown	0	0	0	0	0	0
Adverse events by severity						
Mild	24 (34.8)	46	24 (36.9)	50	48 (35.8)	96
Moderate	12 (17.4)	30	11 (16.9)	13	23 (17.2)	43
Severe	4 (5.8)	7	5 (7.7)	6	9 (6.7)	13
Adverse events by causality						
Not related	11 (15.9)	17	13 (20.0)	29	24 (17.9)	46
Unlikely related	10 (14.5)	13	11 (16.9)	19	21 (15.7)	32
Possibly related	13 (18.8)	35	8 (12.3)	12	21 (15.7)	47
Probably related	8 (11.6)	18	6 (9.2)	9	14 (10.4)	27
Related	0	0	0	0	0	0

Source: CSR 043/SI Table 14.3.3.1

"Based on Agency feedback, severe AEs were reevaluated for seriousness, leading to the identification of 4 AEs that are now considered serious"

m = number of events; n = number of subjects.
Percentages are based on the number of subjects within each treatment group

Table 74. Frequency of adverse events in patients with *Acanthamoeba* keratitis (Study 043, SAS)

	0.8 mg/mL polihexanide + placebo (N=69)		0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=65)		Total (N=134)	
System Organ Class/Preferred Term	n (%)	m	n (%)	m	n (%)	m
Any adverse event	**31 (44.9)**	**83**	**29 (44.6)**	**69**	**60 (44.8)**	**152**
Eye disorders	**23 (33.3)**	**60**	**20 (30.8)**	**41**	**43 (32.1)**	**101**
Eye pain	9 (13.0)	11	7 (10.8)	7	16 (11.9)	18
Ocular hyperaemia	8 (11.6)	9	7 (10.8)	9	15 (11.2)	18
Lacrimation increased	6 (8.7)	6	2 (3.1)	2	8 (6.0)	8
Conjunctival hyperaemia	2 (2.9)	2	3 (4.6)	4	5 (3.7)	6
Eye irritation	1 (1.4)	1	4 (6.2)	4	5 (3.7)	5
Photophobia	2 (2.9)	2	3 (4.6)	3	5 (3.7)	5
Eye inflammation	3 (4.3)	3	1 (1.5)	3	4 (3.0)	6
Corneal epithelium defect	3 (4.3)	3	0	0	3 (2.2)	3
Corneal infiltrates	3 (4.3)	3	0	0	3 (2.2)	3
Eye pruritus	1 (1.4)	1	2 (3.1)	2	3 (2.2)	3
Corneal perforation	1 (1.4)	1	1 (1.5)	1	2 (1.5)	2
Eye discharge	2 (2.9)	2	0	0	2 (1.5)	2
Eye swelling	2 (2.9)	2	0	0	2 (1.5)	2
Keratitis	2 (2.9)	2	0	0	2 (1.5)	2
Ocular discomfort	2 (2.9)	2	0	0	2 (1.5)	2
Vision blurred	1 (1.4)	1	1 (1.5)	1	2 (1.5)	2
Conjunctival oedema	0	0	1 (1.5)	1	1 (0.7)	1
Conjunctival papillae	1 (1.4)	1	0	0	1 (0.7)	1
Corneal neovascularisation	1 (1.4)	1	0	0	1 (0.7)	1
Corneal oedema	0	0	1 (1.5)	1	1 (0.7)	1
Diplopia	1 (1.4)	1	0	0	1 (0.7)	1
Dry eye	1 (1.4)	1	0	0	1 (0.7)	1
Eyelid cyst	0	0	1 (1.5)	1	1 (0.7)	1
Eyelid oedema	1 (1.4)	1	0	0	1 (0.7)	1
Foreign body sensation in eyes	1 (1.4)	1	0	0	1 (0.7)	1
Glaucoma	0	0	1 (1.5)	1	1 (0.7)	1
Punctate keratitis	1 (1.4)	1	0	0	1 (0.7)	1
Scleritis	0	0	1 (1.5)	1	1 (0.7)	1
Ulcerative keratitis	1 (1.4)	1	0	0	1 (0.7)	1
Visual impairment	1 (1.4)	1	0	0	1 (0.7)	1
Infections and infestations	**9 (13.0)**	**9**	**8 (12.3)**	**8**	**17 (12.7)**	**17**
Herpes ophthalmic	1 (1.4)	1	1 (1.5)	1	2 (1.5)	2
Hypopyon	1 (1.4)	1	1 (1.5)	1	2 (1.5)	2
Conjunctivitis	1 (1.4)	1	0	0	1 (0.7)	1
Conjunctivitis viral	1 (1.4)	1	0	0	1 (0.7)	1
Eye infection	1 (1.4)	1	0	0	1 (0.7)	1
Eye infection bacterial	0	0	1 (1.5)	1	1 (0.7)	1
Herpes virus infection	1 (1.4)	1	0	0	1 (0.7)	1
Influenza	0	0	1 (1.5)	1	1 (0.7)	1
Keratitis bacterial	0	0	1 (1.5)	1	1 (0.7)	1
Lower respiratory tract infection	0	0	1 (1.5)	1	1 (0.7)	1
Ophthalmic herpes simplex	0	0	1 (1.5)	1	1 (0.7)	1
Oral herpes	1 (1.4)	1	0	0	1 (0.7)	1

Pharyngitis	1 (1.4)	1	0	0	1 (0.7)	1
Upper respiratory tract infection	1 (1.4)	1	0	0	1 (0.7)	1
Urinary tract infection	0	0	1 (1.5)	1	1 (0.7)	1
General disorders and administration site conditions	**4 (5.8)**	**5**	**5 (7.7)**	**6**	**9 (6.7)**	**11**
Condition aggravated	1 (1.4)	2	1 (1.5)	1	2 (1.5)	3
Application site discomfort	1 (1.4)	1	1 (1.5)	1	2 (1.5)	2
Application site pain	1 (1.4)	1	1 (1.5)	1	2 (1.5)	2
Application site pruritus	0	0	1 (1.5)	1	1 (0.7)	1
Drug intolerance	1 (1.4)	1	0	0	1 (0.7)	1
Fatigue	0	0	1 (1.5)	1	1 (0.7)	1
Pyrexia	0	0	1 (1.5)	1	1 (0.7)	1
Gastrointestinal disorders	**2 (2.9)**	**2**	**3 (4.6)**	**3**	**5 (3.7)**	**5**
Nausea	1 (1.4)	1	2 (3.1)	2	3 (2.2)	3
Abdominal discomfort	1 (1.4)	1	0	0	1 (0.7)	1
Abdominal pain upper	0	0	1 (1.5)	1	1 (0.7)	1
Injury, poisoning and procedural complications	**2 (2.9)**	**2**	**2 (3.1)**	**3**	**4 (3.0)**	**5**
Persistent corneal epithelial defect	0	0	1 (1.5)	2	1 (0.7)	2
Eye injury	0	0	1 (1.5)	1	1 (0.7)	1
Joint dislocation	1 (1.4)	1	0	0	1 (0.7)	1
Toxicity to various agents	1 (1.4)	1	0	0	1 (0.7)	1
Nervous system disorders	**1 (1.4)**	**1**	**3 (4.6)**	**3**	**4 (3.0)**	**4**
Dizziness	0	0	1 (1.5)	1	1 (0.7)	1
Headache	1 (1.4)	1	0	0	1 (0.7)	1
Horner's syndrome	0	0	1 (1.5)	1	1 (0.7)	1
Sciatica	0	0	1 (1.5)	1	1 (0.7)	1
Psychiatric disorders	**0**	**0**	**2 (3.1)**	**2**	**2 (1.5)**	**2**
Anxiety	0	0	1 (1.5)	1	1 (0.7)	1
Insomnia	0	0	1 (1.5)	1	1 (0.7)	1
Respiratory, thoracic and mediastinal disorders	**1 (1.4)**	**1**	**1 (1.5)**	**1**	**2 (1.5)**	**2**
Epistaxis	0	0	1 (1.5)	1	1 (0.7)	1
Oropharyngeal pain	1 (1.4)	1	0	0	1 (0.7)	1
Investigations	**1 (1.4)**	**2**	**0**	**0**	**1 (0.7)**	**2**
Blood cholesterol increased	1 (1.4)	1	0	0	1 (0.7)	1
Blood triglycerides increased	1 (1.4)	1	0	0	1 (0.7)	1
Pregnancy, puerperium and perinatal conditions	**0**	**0**	**1 (1.5)**	**1**	**1 (0.7)**	**1**
Pregnancy	0	0	1 (1.5)	1	1 (0.7)	1
Renal and urinary disorders	**0**	**0**	**1 (1.5)**	**1**	**1 (0.7)**	**1**
Haematuria	0	0	1 (1.5)	1	1 (0.7)	1
Surgical and medical procedures	**1 (1.4)**	**1**	**0**	**0**	**1 (0.7)**	**1**
Corneal transplant	1 (1.4)	1	0	0	1 (0.7)	1

Source: CSR 043/SI Table 14.3.3.2
m = number of events; n = number of subjects.
Adverse events are coded according to MedDRA 20.0-24.0.
Percentages are based on the number of subjects within each treatment group

Table 75. Adverse events by severity in patients with *Acanthamoeba* keratitis (Study 043, SAS)

	0.8 mg/mL polihexanide + placebo (N=69)						0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=65)					
System Organ Class	Mild		Moderate		Severe		Mild		Moderate		Severe	
Preferred Term	n (%)	m	n (%)	m	n (%)	m	n (%)	m	n (%)	m	n (%)	m
Any adverse event	**24 (34.8)**	**46**	**12 (17.4)**	**30**	**4 (5.8)**	**7**	**24 (36.9)**	**50**	**11 (16.9)**	**13**	**5 (7.7)**	**6**
Eye disorders	**16 (23.2)**	**30**	**12 (17.4)**	**27**	**2 (2.9)**	**3**	**16 (24.6)**	**32**	**4 (6.2)**	**5**	**4 (6.2)**	**4**
Eye pain	6 (8.7)	6	3 (4.3)	4	1 (1.4)	1	4 (6.2)	4	1 (1.5)	1	2 (3.1)	2
Ocular hyperaemia	4 (5.8)	4	5 (7.2)	5	0	0	6 (9.2)	8	1 (1.5)	1	0	0
Lacrimation increased	4 (5.8)	4	2 (2.9)	2	0	0	2 (3.1)	2	0	0	0	0
Conjunctival hyperaemia	1 (1.4)	1	1 (1.4)	1	0	0	3 (4.6)	4	0	0	0	0
Eye inflammation	1 (1.4)	1	2 (2.9)	2	0	0	1 (1.5)	1	1 (1.5)	2	0	0
Eye irritation	1 (1.4)	1	0	0	0	0	4 (6.2)	4	0	0	0	0
Photophobia	0	0	2 (2.9)	2	0	0	3 (4.6)	3	0	0	0	0
Corneal epithelium defect	2 (2.9)	2	1 (1.4)	1	0	0	0	0	0	0	0	0
Corneal infiltrates	0	0	3 (4.3)	3	0	0	0	0	0	0	0	0
Eye pruritus	1 (1.4)	1	0	0	0	0	2 (3.1)	2	0	0	0	0
Corneal perforation	0	0	0	0	1 (1.4)	1	0	0	0	0	1 (1.5)	1
Eye discharge	1 (1.4)	1	1 (1.4)	1	0	0	0	0	0	0	0	0
Eye swelling	1 (1.4)	1	1 (1.4)	1	0	0	0	0	0	0	0	0
Keratitis	0	0	2 (2.9)	2	0	0	0	0	0	0	0	0
Ocular discomfort	1 (1.4)	1	1 (1.4)	1	0	0	0	0	0	0	0	0
Vision blurred	1 (1.4)	1	0	0	0	0	1 (1.5)	1	0	0	0	0
Conjunctival oedema	0	0	0	0	0	0	1 (1.5)	1	0	0	0	0
Conjunctival papillae	0	0	1 (1.4)	1	0	0	0	0	0	0	0	0
Corneal neovascularisation	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0
Corneal oedema	0	0	0	0	0	0	0	0	1 (1.5)	1	0	0
Diplopia	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0
Dry eye	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0
Eyelid cyst	0	0	0	0	0	0	1 (1.5)	1	0	0	0	0
Eyelid oedema	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0
Foreign body sensation in eyes	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0
Glaucoma	0	0	0	0	0	0	1 (1.5)	1	0	0	0	0
Punctate keratitis	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0
Scleritis	0	0	0	0	0	0	0	0	0	0	1 (1.5)	1
Ulcerative keratitis	0	0	1 (1.4)	1	0	0	0	0	0	0	0	0
Visual impairment	0	0	0	0	1 (1.4)	1	0	0	0	0	0	0
Infections and infestations	**7 (10.1)**	**7**	**1 (1.4)**	**1**	**1 (1.4)**	**1**	**4 (6.2)**	**4**	**3 (4.6)**	**3**	**1 (1.5)**	**1**
Herpes ophthalmic	1 (1.4)	1	0	0	0	0	1 (1.5)	1	0	0	0	0
Hypopyon	0	0	0	0	1 (1.4)	1	0	0	1 (1.5)	1	0	0
Conjunctivitis	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0
Conjunctivitis viral	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0
Eye infection	0	0	1 (1.4)	1	0	0	0	0	0	0	0	0
Eye infection bacterial	0	0	0	0	0	0	1 (1.5)	1	0	0	0	0
Herpes virus infection	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0

Influenza	0	0	0	0	0	0	1 (1.5)	1	0	0	0	0
Keratitis bacterial	0	0	0	0	0	0	0	0	0	0	1 (1.5)	1
Lower respiratory tract infection	0	0	0	0	0	0	0	0	1 (1.5)	1	0	0
Ophthalmic herpes simplex	0	0	0	0	0	0	1 (1.5)	1	0	0	0	0
Oral herpes	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0
Pharyngitis	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0
Upper respiratory tract infection	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0
Urinary tract infection	0	0	0	0	0	0	0	0	1 (1.5)	1	0	0
General disorders and administration site conditions	**2 (2.9)**	**2**	**1 (1.4)**	**1**	**1 (1.4)**	**2**	**4 (6.2)**	**5**	**1 (1.5)**	**1**	**0**	**0**
Condition aggravated	0	0	0	0	1 (1.4)	2	0	0	1 (1.5)	1	0	0
Application site discomfort	1 (1.4)	1	0	0	0	0	1 (1.5)	1	0	0	0	0
Application site pain	1 (1.4)	1	0	0	0	0	1 (1.5)	1	0	0	0	0
Application site pruritus	0	0	0	0	0	0	1 (1.5)	1	0	0	0	0
Drug intolerance	0	0	1 (1.4)	1	0	0	0	0	0	0	0	0
Fatigue	0	0	0	0	0	0	1 (1.5)	1	0	0	0	0
Pyrexia	0	0	0	0	0	0	1 (1.5)	1	0	0	0	0
Gastrointestinal disorders	**2 (2.9)**	**2**	**0**	**0**	**0**	**0**	**3 (4.6)**	**3**	**0**	**0**	**0**	**0**
Nausea	1 (1.4)	1	0	0	0	0	2 (3.1)	2	0	0	0	0
Abdominal discomfort	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0
Abdominal pain upper	0	0	0	0	0	0	1 (1.5)	1	0	0	0	0
Injury, poisoning and procedural complications	**1 (1.4)**	**1**	**1 (1.4)**	**1**	**0**	**0**	**0**	**0**	**2 (3.1)**	**2**	**1 (1.5)**	**1**
Persistent corneal epithelial defect	0	0	0	0	0	0	0	0	1 (1.5)	1	1 (1.5)	1
Eye injury	0	0	0	0	0	0	0	0	1 (1.5)	1	0	0
Joint dislocation	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0
Toxicity to various agents	0	0	1 (1.4)	1	0	0	0	0	0	0	0	0
Nervous system disorders	**1 (1.4)**	**1**	**0**	**0**	**0**	**0**	**2 (3.1)**	**2**	**1 (1.5)**	**1**	**0**	**0**
Dizziness	0	0	0	0	0	0	1 (1.5)	1	0	0	0	0
Headache	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0
Horner's syndrome	0	0	0	0	0	0	1 (1.5)	1	0	0	0	0
Sciatica	0	0	0	0	0	0	0	0	1 (1.5)	1	0	0
Psychiatric disorders	**0**	**0**	**0**	**0**	**0**	**0**	**1 (1.5)**	**1**	**1 (1.5)**	**1**	**0**	**0**
Anxiety	0	0	0	0	0	0	1 (1.5)	1	0	0	0	0
Insomnia	0	0	0	0	0	0	0	0	1 (1.5)	1	0	0
Respiratory, thoracic and mediastinal disorders	**1 (1.4)**	**1**	**0**	**0**	**0**	**0**	**1 (1.5)**	**1**	**0**	**0**	**0**	**0**
Epistaxis	0	0	0	0	0	0	1 (1.5)	1	0	0	0	0
Oropharyngeal pain	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0
Investigations	**1 (1.4)**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Blood cholesterol increased	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0
Blood triglycerides increased	1 (1.4)	1	0	0	0	0	0	0	0	0	0	0
Pregnancy, puerperium and perinatal conditions	**0**	**0**	**0**	**0**	**0**	**0**	**1 (1.5)**	**1**	**0**	**0**	**0**	**0**
Pregnancy	0	0	0	0	0	0	1 (1.5)	1	0	0	0	0
Renal and urinary disorders	**0**	**0**	**0**	**0**	**0**	**0**	**1 (1.5)**	**1**	**0**	**0**	**0**	**0**
Haematuria	0	0	0	0	0	0	1 (1.5)	1	0	0	0	0
Surgical and medical procedures	**0**	**0**	**0**	**0**	**1 (1.4)**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Corneal transplant	0	0	0	0	1 (1.4)	1	0	0	0	0	0	0

Source: CSR 043/SI Table 14.3.3.4

m = number of events; n = number of subjects.

Adverse events are coded according to MedDRA 20.0-24.0.

Percentages are based on the number of subjects within each treatment group

Table 76. Adverse events by causality in patients with *Acanthamoeba* keratitis treated with 0.8 mg/ml polihexanide (Study 043, SAS)

	0.8 mg/mL polihexanide + placebo (N=69)									
	Not related		Unlikely related		Possibly related		Probably related		Related	
System Organ Class/Preferred Term	n (%)	m	n (%)	m	n (%)	m	n (%)	m	n (%)	m
Any adverse event	**11 (15.9)**	**17**	**10 (14.5)**	**13**	**13 (18.8)**	**35**	**8 (11.6)**	**18**	**0**	**0**
Eye disorders	**3 (4.3)**	**5**	**8 (11.6)**	**11**	**12 (17.4)**	**28**	**7 (10.1)**	**16**	**0**	**0**
Eye pain	1 (1.4)	1	2 (2.9)	2	3 (4.3)	4	4 (5.8)	4	0	0
Ocular hyperaemia	0	0	1 (1.4)	1	5 (7.2)	5	2 (2.9)	3	0	0
Lacrimation increased	1 (1.4)	1	2 (2.9)	2	1 (1.4)	1	2 (2.9)	2	0	0
Corneal epithelium defect	1 (1.4)	1	0	0	0	0	2 (2.9)	2	0	0
Corneal infiltrates	0	0	0	0	2 (2.9)	2	1 (1.4)	1	0	0
Eye inflammation	0	0	1 (1.4)	1	2 (2.9)	2	0	0	0	0
Conjunctival hyperaemia	1 (1.4)	1	0	0	1 (1.4)	1	0	0	0	0
Eye discharge	0	0	0	0	1 (1.4)	1	1 (1.4)	1	0	0
Eye swelling	0	0	0	0	2 (2.9)	2	0	0	0	0
Keratitis	0	0	1 (1.4)	1	1 (1.4)	1	0	0	0	0
Ocular discomfort	0	0	0	0	1 (1.4)	1	1 (1.4)	1	0	0
Photophobia	0	0	1 (1.4)	1	1 (1.4)	1	0	0	0	0
Conjunctival papillae	0	0	0	0	0	0	1 (1.4)	1	0	0
Corneal neovascularisation	0	0	1 (1.4)	1	0	0	0	0	0	0
Corneal perforation	0	0	0	0	1 (1.4)	1	0	0	0	0
Diplopia	0	0	1 (1.4)	1	0	0	0	0	0	0
Dry eye	0	0	0	0	1 (1.4)	1	0	0	0	0
Eye irritation	0	0	0	0	1 (1.4)	1	0	0	0	0
Eye pruritus	0	0	0	0	1 (1.4)	1	0	0	0	0
Eyelid oedema	1 (1.4)	1	0	0	0	0	0	0	0	0
Foreign body sensation in eyes	0	0	0	0	1 (1.4)	1	0	0	0	0
Punctate keratitis	0	0	0	0	0	0	1 (1.4)	1	0	0
Ulcerative keratitis	0	0	0	0	1 (1.4)	1	0	0	0	0
Vision blurred	0	0	1 (1.4)	1	0	0	0	0	0	0
Visual impairment	0	0	0	0	1 (1.4)	1	0	0	0	0
Infections and infestations	**6 (8.7)**	**6**	**1 (1.4)**	**1**	**2 (2.9)**	**2**	**0**	**0**	**0**	**0**
Conjunctivitis	0	0	0	0	1 (1.4)	1	0	0	0	0
Conjunctivitis viral	1 (1.4)	1	0	0	0	0	0	0	0	0
Eye infection	0	0	0	0	1 (1.4)	1	0	0	0	0
Herpes ophthalmic	1 (1.4)	1	0	0	0	0	0	0	0	0
Herpes virus infection	1 (1.4)	1	0	0	0	0	0	0	0	0
Hypopyon	0	0	1 (1.4)	1	0	0	0	0	0	0
Oral herpes	1 (1.4)	1	0	0	0	0	0	0	0	0
Pharyngitis	1 (1.4)	1	0	0	0	0	0	0	0	0
Upper respiratory tract infection	1 (1.4)	1	0	0	0	0	0	0	0	0
General disorders and administration site conditions	**0**	**0**	**0**	**0**	**2 (2.9)**	**3**	**2 (2.9)**	**2**	**0**	**0**
Condition aggravated	0	0	0	0	1 (1.4)	2	0	0	0	0
Application site discomfort	0	0	0	0	1 (1.4)	1	0	0	0	0
Application site pain	0	0	0	0	0	0	1 (1.4)	1	0	0
Drug intolerance	0	0	0	0	0	0	1 (1.4)	1	0	0
Gastrointestinal disorders	**2 (2.9)**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Abdominal discomfort	1 (1.4)	1	0	0	0	0	0	0	0	0
Nausea	1 (1.4)	1	0	0	0	0	0	0	0	0
Injury, poisoning and procedural complications	**1 (1.4)**	**1**	**0**	**0**	**1 (1.4)**	**1**	**0**	**0**	**0**	**0**
Joint dislocation	1 (1.4)	1	0	0	0	0	0	0	0	0
Toxicity to various agents	0	0	0	0	1 (1.4)	1	0	0	0	0
Investigations	**1 (1.4)**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Blood cholesterol increased	1 (1.4)	1	0	0	0	0	0	0	0	0
Blood triglycerides increased	1 (1.4)	1	0	0	0	0	0	0	0	0
Nervous system disorders	**0**	**0**	**1 (1.4)**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Headache	0	0	1 (1.4)	1	0	0	0	0	0	0
Respiratory, thoracic and mediastinal disorders	**1 (1.4)**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Oropharyngeal pain	1 (1.4)	1	0	0	0	0	0	0	0	0
Surgical and medical procedures	**0**	**0**	**0**	**0**	**1 (1.4)**	**1**	**0**	**0**	**0**	**0**
Corneal transplant	0	0	0	0	1 (1.4)	1	0	0	0	0

Source: CSR 043/SI Table 14.3.3.5.1
m = number of events; n = number of subjects.
Adverse events are coded according to MedDRA 20.0-24.0.
Percentages are based on the number of subjects within each treatment group

Table 77. Adverse events by causality in patients with *Acanthamoeba* keratitis treated with 0.2 mg/ml polihexanide plus 1.0 mg/ml propamidine (Study 043, SAS)

	0.2 mg/mL polihexanide + 1.0 mg/mL Propamidine (N=65)									
	Not related		Unlikely related		Possibly related		Probably related		Related	
System Organ Class/Preferred Term	n (%)	m	n (%)	m	n (%)	m	n (%)	m	n (%)	m
Any adverse event	**13 (20.0)**	**29**	**11 (16.9)**	**19**	**8 (12.3)**	**12**	**6 (9.2)**	**9**	**0**	**0**
Eye disorders	**6 (9.2)**	**13**	**6 (9.2)**	**12**	**6 (9.2)**	**9**	**5 (7.7)**	**7**	**0**	**0**
Ocular hyperaemia	1 (1.5)	3	3 (4.6)	3	2 (3.1)	2	1 (1.5)	1	0	0
Eye pain	3 (4.6)	3	2 (3.1)	2	2 (3.1)	2	0	0	0	0
Eye irritation	0	0	0	0	3 (4.6)	3	1 (1.5)	1	0	0
Conjunctival hyperaemia	1 (1.5)	1	0	0	0	0	2 (3.1)	3	0	0
Photophobia	1 (1.5)	1	2 (3.1)	2	0	0	0	0	0	0
Eye inflammation	1 (1.5)	2	0	0	0	0	1 (1.5)	1	0	0
Eye pruritus	0	0	0	0	2 (3.1)	2	0	0	0	0
Lacrimation increased	1 (1.5)	1	1 (1.5)	1	0	0	0	0	0	0
Conjunctival oedema	1 (1.5)	1	0	0	0	0	0	0	0	0
Corneal oedema	1 (1.5)	1	0	0	0	0	0	0	0	0
Corneal perforation	0	0	1 (1.5)	1	0	0	0	0	0	0
Eyelid cyst	0	0	1 (1.5)	1	0	0	0	0	0	0
Glaucoma	0	0	1 (1.5)	1	0	0	0	0	0	0
Scleritis	0	0	1 (1.5)	1	0	0	0	0	0	0
Vision blurred	0	0	0	0	0	0	1 (1.5)	1	0	0
Infections and infestations	**6 (9.2)**	**6**	**2 (3.1)**	**2**	**0**	**0**	**0**	**0**	**0**	**0**
Eye infection bacterial	1 (1.5)	1	0	0	0	0	0	0	0	0
Herpes ophthalmic	1 (1.5)	1	0	0	0	0	0	0	0	0
Hypopyon	1 (1.5)	1	0	0	0	0	0	0	0	0
Influenza	1 (1.5)	1	0	0	0	0	0	0	0	0
Keratitis bacterial	0	0	1 (1.5)	1	0	0	0	0	0	0
Lower respiratory tract infection	0	0	1 (1.5)	1	0	0	0	0	0	0
Ophthalmic herpes simplex	1 (1.5)	1	0	0	0	0	0	0	0	0
Urinary tract infection	1 (1.5)	1	0	0	0	0	0	0	0	0
General disorders and administration site conditions	**2 (3.1)**	**3**	**1 (1.5)**	**1**	**1 (1.5)**	**1**	**1 (1.5)**	**1**	**0**	**0**
Application site discomfort	0	0	0	0	1 (1.5)	1	0	0	0	0
Application site pain	1 (1.5)	1	0	0	0	0	0	0	0	0
Application site pruritus	0	0	0	0	0	0	1 (1.5)	1	0	0
Condition aggravated	0	0	1 (1.5)	1	0	0	0	0	0	0
Fatigue	1 (1.5)	1	0	0	0	0	0	0	0	0
Pyrexia	1 (1.5)	1	0	0	0	0	0	0	0	0
Gastrointestinal disorders	**2 (3.1)**	**2**	**1 (1.5)**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Nausea	1 (1.5)	1	1 (1.5)	1	0	0	0	0	0	0
Abdominal pain upper	1 (1.5)	1	0	0	0	0	0	0	0	0
Injury, poisoning and procedural complications	**1 (1.5)**	**1**	**0**	**0**	**1 (1.5)**	**1**	**1 (1.5)**	**1**	**0**	**0**
Persistent corneal epithelial defect	0	0	0	0	1 (1.5)	1	1 (1.5)	1	0	0
Eye injury	1 (1.5)	1	0	0	0	0	0	0	0	0
Nervous system disorders	**1 (1.5)**	**1**	**2 (3.1)**	**2**	**0**	**0**	**0**	**0**	**0**	**0**
Dizziness	0	0	1 (1.5)	1	0	0	0	0	0	0
Horner's syndrome	0	0	1 (1.5)	1	0	0	0	0	0	0
Sciatica	1 (1.5)	1	0	0	0	0	0	0	0	0
Psychiatric disorders	**2 (3.1)**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Anxiety	1 (1.5)	1	0	0	0	0	0	0	0	0
Insomnia	1 (1.5)	1	0	0	0	0	0	0	0	0
Pregnancy, puerperium and perinatal conditions	**1 (1.5)**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Pregnancy	1 (1.5)	1	0	0	0	0	0	0	0	0
Renal and urinary disorders	**0**	**0**	**1 (1.5)**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Haematuria	0	0	1 (1.5)	1	0	0	0	0	0	0
Respiratory, thoracic, and mediastinal disorders	**0**	**0**	**0**	**0**	**1 (1.5)**	**1**	**0**	**0**	**0**	**0**
Epistaxis	0	0	0	0	1 (1.5)	1	0	0	0	0

Source: CSR 043/SI Table 14.3.3.5.2

m = number of events; n = number of subjects.
Adverse events are coded according to MedDRA 20.0-24.0.
Percentages are based on the number of subjects within each treatment group

Ocular safety assessments:

Intraocular pressure: Intraocular pressure was measured at all visits during the study. Of the 69 subjects that received at least one dose of 0.8 mg/ml polihexanide + placebo, 3 subjects had at least one increased intraocular pressure (> 21 mm Hg) measurement. Of the 65 subjects that received at least one dose of 0.2 mg/ml polihexanide + 1 mg/ml propamidine, 4 subjects had at least one increased intraocular pressure (> 21 mm Hg) measurement.

Opthalmoscopy: Ophthalmoscopy was performed at the baseline visit before first study drug application and at the follow-up visit. It was also performed at any time in case the pupil tests or intraocular pressure were abnormal. No subject had abnormal vitreous, macula, and choroid at the end of the study. One subject in 0.2 mg/ml + 1 mg propamidine treatment group had abnormal CS retina and no subject in 0.8 mg/ml + placebo treatment group had any abnormal retina.

2.6.8.3. Serious adverse event/deaths/other significant events

No severe AEs and no SAEs, including severe life-threatening or blinding events, occurred in healthy volunteers.

No AEs leading to death occurred in patients with *Acanthamoeba* keratitis. 13 AEs in 9 patients were classified as severe. 7 occurred in 4 patients (5.8%) treated with 0.8 mg/mL polihexanide + placebo and 6 occurred 5 patients (7.7%) treated with 0.2 mg/mL polihexanide plus 1.0 mg/mL propamidine. The PTs related to these events were eye pain, corneal perforation (2x), corneal damage resulting in cornea transplant, condition aggravated, scleritis, bacterial keratitis, persistent epithelial defect, hypopyon and deteriorating vision. The following four severe events were further classified as serious according to CIOMS criteria: corneal perforation (1 case in the 0.8 mg/mL polihexanide arm, 1 case in the combination therapy arm), corneal transplant (1 case in the 0.8 mg/mL polihexanide arm), and visual impairment (1 case in the 0.8 mg/mL polihexanide arm).

Table 78. List of severe AEs occurred in the Study 043/SI that meet the CIOMS criteria for SAEs

	AE classified as "severe"	0.8 mg/mL polihexanide + placebo (number of events)	0.2 mg/mL polihexanide + 1 mg/mL propamidine (number of events)		
Patient ID no.	Preferred term			CIOMS criteria for SAEs	CIOMS Criteria
11-02, 22-10, 11-66	Eye pain	1	2	NO	Not applicable
11-24, 11-32	**Corneal perforation**	**1**	**1**	**YES**	**Hospitalization/ prolonged hospitalization**
11-22	Scleritis	0	1	NO	Not applicable
11-66	**Visual impairment**	**1**	0	**YES**	**Disabling**
22-15	Hypopyon	1	0	NO	Not applicable
11-32	Bacterial keratitis	0	1	NO	Not applicable
11-41	Condition aggravated	2	0	NO	Not applicable
11-54	Persistent corneal defect	0	1	NO	Not applicable
11-24	**Corneal transplant**	**1**	0	**YES**	**Hospitalization/ prolonged hospitalization**
TOTAL		7	6		

2.6.8.4. Laboratory findings

Study 042/SI

Clinical laboratory parameters (clinical chemistry, haematology, and urinalysis) were evaluated at each visit. Any abnormal laboratory value that constituted an SAE or led to discontinuation of the IMP was recorded as an AE. Haematology, biochemistry and urinalysis data were summarized with descriptive statistics for changes from baseline to the end-of-study visit. There were no clinically significant or meaningful changes in mean values over time in any of the laboratory variables. There were isolated laboratory values below or above reference ranges across all dose groups; however, no out of range values were assessed as clinically significant by the Investigator.

Vital signs were evaluated at each visit. There were no clinically significant changes in the mean values of systolic or diastolic blood pressure or pulse during the study in any treatment group. There were no clinically significant changes in mean values over time in any variables.

Study 043/SI

Clinical laboratory parameters were evaluated at baseline and at the end of the study for haematology and clinical chemistry. Abnormal test finding, e.g., abnormal laboratory analysis results were recorded as an AE. Descriptive statistics for haematology and clinical chemistry parameters were similar between the 0.8 mg/mL polihexanide + placebo group compared to the 0.2 mg/mL polihexanide + 1.0 mg/mL propamidine group. Shifts in haematology parameters and clinical chemistry parameters from normal to abnormal (including whether clinically significant or not clinically significant) between baseline and end of the study were recorded. No shifts from normal at baseline to clinically significant abnormal values at end of study were observed. No clinically significant shifts from normal at baseline to clinically significant abnormal values at end of study were observed.

Urinalysis: Patient rates of specific gravity and of pH values were similar at baseline and end of the study for both treatment groups; and shifts from baseline to end of the study for presence of protein, glucose, ketones, urobilinogen, erythrocytes, leukocytes were similar between treatment groups. Shifts in urinalysis parameters from normal to abnormal (including whether clinically significant or not clinically significant) between baseline and end of the study were recorded. A shift in erythrocytes and leukocytes from normal at baseline to clinically significant abnormal values at end of the study was observed for 1 patient each in the 0.2 mg/mL polihexanide + 1.0 mg/ml propamidine group. Otherwise, there were no shifts from normal at baseline to clinically significant abnormal values at end of the study.

Vital Signs including diastolic and systolic blood pressure, pulse rate and body temperature were only measured at baseline.

2.6.8.5. In vitro biomarker test for patient selection for safety

Not applicable.

2.6.8.6. Safety in special populations

No safety data are available for special populations. According to the applicant, there is no evidence that the frequency of AE is related to age or gender. Due to the local route of administration, it is unlikely that patients with hepatic or renal impairment require special considerations. Pregnant and breast-feeding females were excluded from the study and therefore the use of Akantior during pregnancy and in lactating women is only recommended if the benefits outweigh the risks.

Table 79. Safety in either patient group by age group (Safety Analysis Set)

MedDRA Terms	Age <65 n (%)	Age 65-74 n (%)	Age 75-84 n (%)	Age 85+ n (%)
Total patients	129 (96.3)	5 (3.7)	0	0
Total AEs	55 (42.6)	5 (100)	0	0
Serious AE	3 (2.3)	0	0	0
- Fatal	0	0	0	0
- Hospitalization/prolong existing hospitalization	2 (1.6)	0	0	0
- Life-threatening	0	0	0	0
- Disability/incapacity	1 (0.8)	0	0	0
- Other (medically significant)	0	0	0	0
AE leading to drop-out	14 (10.8)	0	0	0
Psychiatric disorders	2 (1.6)	0	0	0
Nervous system disorders	4 (3.1)	0	0	0
Accidents and injuries	0	0	0	0
Cardiac disorders	0	0	0	0
Vascular disorders	0	0	0	0
Cerebrovascular disorders	0	0	0	0
Infections and infestations	17 (13.2)	0	0	0
Anticholinergic syndrome	0	0	0	0
Quality of life decreased	0	0	0	0
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures	0	0	0	0

Source: post-hoc statistical analysis (Table Q10.1 and Table Q10.2)

Based on Agency feedback, severe AEs were reevaluated for seriousness, leading to the identification of 4 AEs that are now considered serious.

2.6.8.7. Immunological events

No immunological studies were performed.

2.6.8.8. Safety related to drug-drug interactions and other interactions

No specific drug-interaction studies were performed. Given the ophthalmic method of administration and presumably negligible systemic exposure, the Applicant stated that no systemic drug-drug interactions are expected. According to the Applicant, there were no data available in the scientific literature providing evidence for an interaction between polihexanide and other medicine products.

2.6.8.9. Discontinuation due to adverse events

Study 042/SI:

Table 80. Summary of adverse events leading to premature discontinuation of study drug (dose limiting events) in healthy volunteers (Study 042)

Subject #	Treatment group	AE Preferred Term	Duration (days)	Severity	Causality	Serious Y/N	Outcome
302	0.8 mg/mL polihexanide	Conjunctivitis allergic	14	Mild	Related	N	Recovered/Resolved
303	0.8 mg/mL polihexanide	Vital dye staining cornea present	12	Moderate	Related	N	Recovered/Resolved
		Instillation site dryness	12	Moderate	Related	N	Recovered/Resolved
306	0.8 mg/mL polihexanide	Conjunctivitis allergic	15	Moderate	Related	N	Recovered/Resolved
		Instillation site dryness	11	Moderate	Related	N	Recovered/Resolved
		Vital dye staining cornea present	11	Moderate	Related	N	Recovered/Resolved
307	0.6 mg/mL polihexanide	Conjunctivitis allergic	13	Mild	Related	N	Recovered/Resolved
417	0.6 mg/mL polihexanide	Conjunctival irritation	5	Moderate	Probable	N	Recovered/Resolved
		Instillation site foreign body sensation	1	Moderate	Probable	N	Recovered/Resolved

Source: CSR 042/SI Listing 16.2.1.1 I and Listing 16.2.2.5

Study 043/SI:

Table 81. List of adverse events leading to premature discontinuation of study drug in patients with *Acanthamoeba* keratitis (Study 043)

Subject #	Treatment group	AE Preferred Term	Duration (days)	Severity	Causality	Serious Y/N	Outcome
1117	0.2 mg/mL polihexanide + 1.0 mg/mL propamidine	Eye inflammation	9	Moderate	Probably Related	N	Recovered/ Resolved
1122	0.2 mg/mL polihexanide + 1.0 mg/mL propamidine	Scleritis	191	Severe	Unlikely Related	N	Recovered/ Resolved
1124	0.8 mg/mL polihexanide + placebo	Corneal perforation Corneal transplant	35	Severe	Possibly Related	Y Y	Recovered/ Resolved
1132	0.2 mg/mL polihexanide + 1.0 mg/mL propamidine	Corneal perforation	37	Severe	Unlikely Related	Y	Recovered/ Resolved
1141	0.8 mg/mL polihexanide + placebo	Condition aggravated	Ongoing	Severe	Possibly Related	N	Recovering/ Resolving
1143	0.2 mg/mL polihexanide + 1.0 mg/mL propamidine	Condition aggravated	33	Moderate	Unlikely Related	N	Recovered/ Resolved
1149	0.2 mg/mL polihexanide +	Not coded					

	1.0 mg/mL propamidine						
1154	0.2 mg/mL polihexanide + 1.0 mg/mL propamidine	Persistent corneal epithelial defect	15	Severe	Probably Related	N	Recovered/ Resolved
1166	0.8 mg/mL polihexanide + placebo	Eye pain Visual impairment	Ongoing	Severe	Possibly Related	N Y	Recovered/ Resolved
2118	0.8 mg/mL polihexanide + placebo	Herpes ophthalmic	Ongoing	Mild	Not Related	N	Recovering/ Resolving
2203	0.8 mg/mL polihexanide + placebo	Eye inflammation Eye pain	Ongoing Ongoing	Moderate Moderate	Possibly Related Possibly Related	N N	Unknown Unknown
2211	0.8 mg/mL polihexanide + placebo	Corneal epithelium defect Corneal infiltrates	Ongoing Ongoing	Moderate Moderate	Probably related Probably related	N N	Not recovered/ Not resolved Not recovered/ Not resolved
2215	0.8 mg/mL polihexanide + placebo	Hypopyon	Ongoing	Severe	Unlikely related	N	Unknown
2210	0.2 mg/mL polihexanide + 1.0 mg/mL propamidine	Hypopyon	Ongoing	Moderate	Not Related	N	Not recovered/ Not resolved

Source: CSR 043/SI Listing 16.2.1.1, Listing 16.2.7 (adverse event listings).
Based on Agency feedback, severe AEs were reevaluated for seriousness, leading to the identification of 4 AEs that are now considered serious

2.6.8.10. Post marketing experience

Akantior had not yet received a marketing authorisation at the time of this report, thus no post-marketing data were available.

2.6.9. Discussion on clinical safety

The safety evaluation of Akantior (0.8 mg/mL polihexanide) is based on 27 healthy volunteers (Phase I study 042/SI) exposed for 2 weeks and 69 patients with *Acanthamoeba* keratitis (Phase III study 043/SI) exposed for approximately 35 weeks (max. up to one year).

Globally, the strategy for safety evaluation is reasonable. The number of patients included in the safety database, although not very high in absolute numbers, is considered acceptable due to the rarity of the disease. The duration of the safety follow-up in the Phase III study is also acceptable due to the fast clearance of the ophthalmic administered product. No relevant safety events are expected three months after the last dose of polihexanide. The study design of both studies is adequate for the outlined safety evaluations.

During clinical development of Akantior, three dose levels of polihexanide were evaluated in the Phase I study: 0.4 mg/mL, 0.6 mg/mL and 0.8 mg/mL polihexanide. The dose selected for the Phase III study was 0.8 mg/mL polihexanide. The proportion of subjects reporting TEAEs was higher in the 0.6 mg/mL polihexanide dosing group compared to the other two dosing groups, which might have arisen from the small sample size. The applicant´s decision on choosing the highest dose, 0.8 mg/mL polihexanide for the Phase III study is conclusive and can be followed from a safety perspective, since evaluated doses in study 042/SI showed no dose dependency in the occurrence of AEs and hence performed similarly in terms of safety. In the subsequent pivotal Phase III trial the efficacy and safety of 0.8 mg/mL polihexanide monotherapy in patients with *Acanthamoeba* keratitis were evaluated, compared to polihexanide 0.2 mg/mL + propamidine 1.0 mg/mL. According to the Applicant, the

comparator treatment was identified as the most abundant non-licensed supportive care treatment for *Acanthamoeba* keratitis in a retrospective study (038/SI). Regarding the acceptability of the comparator, please see Clinical Efficacy section.

Patients involved in the clinical development of Akantior can most likely be considered representative of the European population. Despite the fact that there is no data about the ethnic origin of the patients in the safety reports, it is considered highly unlikely that the majority of patients would be of non-European ancestry and no further concern is raised on that matter.

No specific clinical studies were carried out in special populations. In the Phase III study, three adolescent patients (12 – 18 years) were included and exposed to 0.8 mg/ml polihexanide. The infectious disease is the same and adolescent eyes are not anatomically or functionally different from adult eyes in matters of relevance to the disease to be treated. Extrapolation from the older population is therefore considered acceptable.

Moreover, only two patients older than 65 years (66, 73 years) were enrolled and exposed to 0.8 mg/ml polihexanide in the Phase III study. Due to the low number of elderly, no specific subgroup analysis of safety data is requested. There is no data regarding the use of polihexanide in pregnant and breast-feeding women.

In both clinical studies AEs were collected and assessed for severity, seriousness, onset, duration, therapy (if any) outcome, and likelihood of drug causation (relation). All AEs were followed-up until they were resolved, or the investigator assesses them as persistent.

Overall, there are no major safety concerns about multiple dosing of polihexanide ophthalmic solution in healthy volunteers. No severe AEs and no SAEs, including severe life-threatening or blinding events, occurred in the study. No significant differences in the frequency of TEAEs in different treatment groups compared to placebo was detected. There is no clear dose-response relationship in terms of AEs and doses up to 0.8 mg/mL polihexanide. Observations in healthy volunteers did not reveal any serious safety concern associated with 0.8 mg/mL polihexanide treatment. The majority of all TEAEs occurred within the SOCs Eye disorders, General disorders and administration site condition and Investigations.

Of note, a dose-dependent effect in vital staining of the cornea (suggesting a corneal defect) in healthy volunteers was identified. The applicant was asked to discuss whether treatment with polihexanide could enhance disease severity. In the response to this concern, the Applicant explained that corneal staining returned to baseline within 1 week after discontinuation of treatment in all subjects, apart from two subjects in whom resolution took 2 weeks. Vital staining has not been performed in the Applicant's pivotal Phase III trial in patients. However, there is no indication that toxicity was a substantial clinical issue for either treatment (0.8mg/ml polihexanide + placebo or 0.2 mg/ml polihexanide + 1 mg/ml propamidine). Only one subject in the 0.8mg/ml polihexanide arm prematurely discontinued the study after 9 months on treatment due to treatment toxicity. No toxicity was observed in any of the subjects with a medical cure. Thus, there is no substantial evidence that treatment with polihexanide triggers accelerated disease progression.

In principle, the safety profile of 0.8 mg/ml polihexanide observed in healthy volunteers seems acceptable, since adverse events were rather common but mostly mild and no SAEs occurred. No major safety concerns were detected in any of the dosing groups when compared to placebo. In total 78.3% of TEAEs were assessed as either possibly related, probably related or related to IMP. The grading of relatedness to IMP (by SOC, not individual AE) is in general considered acceptable. No safety concern persists in that regard.

In patients with *Acanthamoeba* keratitis the overall safety profile of 0.8 mg/ml polihexanide + placebo is similar to the safety profile of the comparator treatment 0.2 mg/ml polihexanide + 1 mg/ml propamidine. The proportion of subjects with any AE was similar in both treatment groups. Of note, in

both treatment arms the same active substance was part of the treatment regimen and therefore a similar AE reporting is not unexpected. In general, the reporting of AEs was rather common, their intensity mild or moderate. No deaths were reported in the study. 13 AEs in 9 patients were classified as severe. Severe AEs were evenly distributed between both treatment arms, although due to the unclear risk profile of the comparator the impact of this even distribution upon the B/R of polihexanide is unclear. Individual severe events showed a higher incidence in the 0.8 mg/ml polihexanide + placebo arm. After requested re-evaluation, four of the 13 reported severe events were further classified as serious according to CIOMS criteria: corneal perforation (1 case in the 0.8 mg/mL polihexanide + placebo arm, 1 case in the combination therapy arm), corneal transplant (1 case in the 0.8 mg/mL polihexanide + placebo arm), and visual impairment (1 case in the 0.8 mg/mL polihexanide +placebo arm). No AEs were assessed as certainly related to the study treatment. Possibly or probably related AEs occurred in 30.4% of patients treated with 0.8 mg/mL polihexanide + placebo and 21.5% of patients treated with 0.2 mg/mL polihexanide + 1.0 mg/mL propamidine. The grading regarding relatedness to treatment can widely be followed. Possibly or probably related AEs were adequately reflected in section 4.8 in the SmPC.

No relevant systemic AEs were identified in healthy subjects and patients with AK. There were only a few isolated non-eye-disorder-related (systemic) AEs in both populations that are not expected to be related to the ophthalmic administered IMP.

Some safety events were identified in the Phase III study that might be of relevance for the benefit/risk evaluation:

- In the 0.8 mg/mL polihexanide + placebo arm two corneal transplantation and one corneal perforation event occurred in two patients. In the comparator arm only one patient experienced a corneal transplantation event. Considering the low number of patients in the study, the frequency of having a corneal perforation or transplantation event is three times higher in the 0.8 mg/mL polihexanide + placebo arm than in the comparator arm. Moreover, one subject in the 0.8 mg/ml polihexanide + placebo arm experienced a mild event of corneal neovascularization. Although corneal perforation and/or corneal transplant are part of the natural history of untreated or resistant *Acanthamoeba* keratitis this is of concern and it needs to be elaborated whether those events were rather caused by polihexanide treatment or disease progression, which could potentially be associated with lack of efficacy of the study treatment. Based on the provided discussion and information pertaining to the corneal perforation/transplantation events, of whom two were considered secondary to bacterial keratitis, there is no substantial evidence that those events were caused by polihexanide treatment.

- In the 0.8 mg/mL polihexanide + placebo arm four patients had to be given a repeated dose (19 days intensive treatment course) after experiencing worsening of the disease. Of note, 3 out of these 4 patients had reported steroid use before treatment. There is, hence, a theoretical concern, that previous steroid use could potentially be associated with worse outcome regarding efficacy (please refer to Non-Clinic and Clinical Efficacy).

- In the 0.8 mg/ml polihexanide + placebo arm two patients experienced abnormal intraocular pressure. Since intraocular pressure is an important safety variable when applying topical formulations, it needed to be clarified whether there could be a higher risk for developing abnormal IOP after polihexanide administration. In response to this concern, the Applicant provided a list of patients with at least one IOP measurement >21 mmHg (which is considered abnormal according to EGS 2021). In total, 7 patients (3/69 in the 0.8 mg/ml polihexanide + placebo arm; 4/65 in the 0.2 mg/ml polihexanide + 1 mg/ml propamidine arm) had at least one IOP value >21 mmHg, however at the end of the study all of these 7 patients had an IOP within the normal range. Since the elevated IOP measures were mostly single events (in two patients 2 events each) during several months of

treatment and IOP values were normal at the end of the study, a causal relationship between the use of polihexanide and elevated IOP is considered unlikely. As outlined by the Applicant, elevated IOP may be attributed to disease progression or concomitant topical corticosteroid use since ocular hypertension is a possible complication of AK and also a consequence of the use of corticosteroids.

- Two events of persistent corneal epithelial defect (PED) appeared in one patient in the 0.2 mg/ml polihexanide + 1 mg/ml propamidine treatment group. Since it is not possible to determine whether these AEs were related to polihexanide or propamidine, the Applicant was asked to discuss the possible relationship to polihexanide treatment. Although still not certain, the Applicant argues that the PED is probably more often associated with the preservative benzalkonium chloride (constituent of the formulation in which propamidine is administered). It is noted that PED could be due to preceding corneal injury e.g. corneal damage (corneal staining) which appeared in a dose-dependent manner in healthy volunteers as described above. However, since no PED event occurred in healthy volunteers, and only two events of PED occurred in one AK patient, there is no substantial evidence pointing to a relationship to polihexanide.

- Concurrent eye disorders were balanced across treatment groups, but more cases of PT eye pain were reported in the 0.8 mg/ml polihexanide monotherapy group. The applicant was asked to elaborate whether any data exist that could clarify if eye pain (13.0% and 4.6% for the monotherapy and combination therapy, respectively) was caused by the underlying disease and/or the study treatment. To address this, the temporary association of the eye pain was important to clarify. While the disease itself may indeed cause eye pain as a symptom, this is expected to be more continuously present at least until treatment effect and diminishing of the disease burden. Eye pain caused by the eye drops (which are indeed described after local application of polihexanide, especially post-surgery) were to be expressed in association with application of the eye drops. In response to this concern, the Applicant clarified that while on study treatment, eye pain was reported in a comparable percentage of patients in the two treatment groups, i.e., in 9 patients (13.0%) treated with 0.8 mg/ml polihexanide monotherapy and in 7 patients (10.8%) treated with the combination therapy. The percentages stated above refer to concurrent diseases at baseline, hence could not have been caused by the study treatment. This is acknowledged. Since eye pain is a hallmark of AK and percentages of patients with concurrent eye pain at baseline and percentages of reported eye pain events within the clinical study programme are comparable, it can be assumed that eye pain is rather caused by the underlying disease than by the treatment. This is supported by the provided temporary association of eye pain with the study treatment where it was shown that eye pain events are rather continuously present throughout the study. Further, it is supported by data in healthy volunteers, where only two eye pain events were reported, one in the 0.6 mg/ml polihexanide group and one in the placebo group. Still, a possible causal relation between polihexanide treatment and the onset of eye pain cannot be fully excluded.

The abnormalities detected in the ocular assessments were consistent with the reported TEAEs.

Concerning laboratory evaluations and vital sign assessments, there was no clear pattern of change associated to the study medication in healthy volunteers and AK patients.

No immunological studies and no drug-drug interaction studies have been performed. Given the local route of administration and presumably negligible systemic exposure this could be considered acceptable. Still, the Applicant was asked to present a discussion whether any evidence for hypersensitivity reactions could be identified during the clinical development programme on polihexanide treatment. Since no hypersensitivity/anaphylactic events were reported within the clinical study programme and hypersensitivity reports related to polihexanide, despite its established and long-term use as a disinfectant, are rare, the immunogenic potential of polihexanide seems to be low

and does not give raise to concern for the proposed indication. No issues solely pertaining to safety were identified regarding drug-drug interactions (please also refer to clinical pharmacology).

Furthermore, there were no meaningful clinical findings for AEs leading to premature discontinuation of the study drug in healthy volunteers and patients with *Acanthamoeba* keratitis. Events leading to premature discontinuation of the IMP were evenly distributed between the 0.8 mg/ml polihexanide monotherapy arm and the comparator arm in the Phase III study.

In general, the safety findings in the clinical programme for Akantior are consistent with findings in published studies using ophthalmic polihexanide (different concentrations) in patients with *Acanthamoeba* keratitis.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

2.6.10. Conclusions on the clinical safety

Overall, treatment with polihexanide 0.8mg/ml seems to be well-tolerated. Most reported AEs were of mild or moderate severity.

2.7. Risk Management Plan

2.7.1. Safety concerns

The applicant proposed the following summary of safety concerns in the RMP:

Table SVIII.1: Summary of safety concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	None
Missing information	None

2.7.1.1. Discussion on safety specification

Having considered the data in the safety specification, the PRAC agrees that the safety concerns listed by the applicant are appropriate.

2.7.1.2. Conclusions on the safety specification

Having considered the data in the safety specification

The PRAC agrees that the safety concerns listed by the applicant are appropriate.

2.7.2. Pharmacovigilance plan

Routine pharmacovigilance activities

Routine monitoring is proposed.

Additional pharmacovigilance activities

The are no on-going or planned additional pharmacovigilance studies/activities.

The pharmacovigilance plan was acceptable to the PRAC.

2.7.2.1. Overall conclusions on the PhV Plan

The PRAC, having considered the data submitted, is of the opinion that the proposed post-authorisation PhV development plan is sufficient to identify and characterise the risks of the product.

2.7.3. Risk minimisation measures

Not applicable. No safety concerns have been identified for Akantior.

PRAC outcome

The PRAC noted updated RMP version 0.2 and endorsed the modified list of safety concerns (no risks, no missing information). The PRAC pointed out however that the risk minimisation measures listed in the RMP had to be aligned with the actual safety specification.

2.7.4. Conclusion

The CHMP and PRAC considered that the risk management plan version 0.3 (date of sign-off 5 March 2024) was acceptable.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The active substance is not included in the EURD list and a new entry will be required.

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD). The new EURD list entry will therefore use the EBD to determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Labelling exemptions

A request of translation exemption of the labelling as per Art.63.1 of Directive 2001/83/EC has been submitted by the applicant and has been found partially acceptable by the QRD Group, as follows:

The Group agreed to have an English-only label for the single-dose container, but not for the sachet. The sachet should be provided in the relevant languages as it is used directly by patients.

The labelling subject to translation exemption as per the QRD Group decision above will however be translated in all languages in the Annexes published with the EPAR on EMA website, but the printed materials will only be translated in the language(s) as agreed by the QRD Group.

3. Benefit-Risk Balance

3.1. Therapeutic context

3.1.1. Disease or condition

Akantior is intended to be indicated for the treatment of *Acanthamoeba* keratitis (AK).

Acanthamoeba keratitis is a rare, painful, and sight-threatening infection caused by *Acanthamoeba* species, a family of free-living, ubiquitous protozoa commonly found in water, dust, and soil. This infection results from invasion of ocular tissue through a corneal lesion. The condition is rare (orphan designation) with increasing incidence in past decades and mostly affects subject wearing contact lenses.

3.1.2. Available therapies and unmet medical need

There are no drugs licensed for use in *Acanthamoeba* keratitis and no standard treatment is established across the EU. Thus, patients and ophthalmologists use unlicensed or off-label treatment options. Diamidines and biguanides are considered effective cysticidal anti-amoebic agents. These products are available in the EU (including polihexanide, propamidine, chlorhexidine and hexamidine) and are also used for the ocular treatment of AK, albeit not being specifically approved for this indication.

The lack of a uniform treatment approach across ophthalmology centres in the EU is critically noted. A validated treatment option with proven efficacy and described magnitude of effect could be an improvement for the treatment of AK in the EU.

3.1.3. Main clinical studies

The main clinical evidence derives from the single pivotal phase 3 study 043.

Study 043 was a randomised, double-masked, double-dummy, active-controlled, multi-centre, parallel-group Phase 3 study to evaluate the efficacy, safety, and tolerability of 0.8 mg/ml polihexanide ophthalmic solution compared to 0.2 mg/ml polihexanide + 1 mg/ml propamidine combination therapy used as active comparator in male and female subjects affected by *Acanthamoeba* keratitis.

The aim of 0.8 mg/ml polihexanide is to increase clinical resolution for AK patients (primary outcome was the clinical resolution rate within 12 months) and to reduce the treatment failures seen with 0.2

mg/ml polihexanide + 1 mg/ml propamidine. A total of 130 subjects affected by *Acanthamoeba* keratitis were planned to be assigned on a 1:1 basis. The Safety Analysis Set included 69 subjects that were exposed to 0.8 mg/ml polihexanide and 65 subjects that were exposed to 0.2 mg/ml polihexanide + 1 mg/ml propamidine.

Study planning included the possibility to switch to non-inferiority testing in case the superiority hypothesis was not met. During assessment the Applicant derived an estimate of the treatment effect compared to "untreated" historical control cases, based on a systematic literature review (SLR).

3.2. Favourable effects

The mode of action of polihexanide has been established in a multitude of non-clinical (*in vitro* and *in vivo*) literature studies, including the disruption of cell membranes by electrostatic binding of polihexanide to the cell membranes of trophozoites (causing membrane damage and ultimately cell lysis), and polihexanide-mediated DNA complexation and chromosome condensation.

In the pivotal phase III study, the difference in proportion of clinical resolution rate is -0.04 with 95% CI (-0.16, 0.09) in the FAS and -0.02 with 95% CI (-0.15, 0.10) in the PP. The lower limit of the 95% CI is above the pre-defined non-inferiority margin of 20%, i.e. polihexanide +placebo is not more than 20% worse than polihexanide +propamidine.

For either treatment arm, improvement from baseline is evident for the primary outcome measure and was demonstrated for secondary measures.

Based on identified observational and case studies, the clinical resolution rate without surgery for patients not treated with antiamoebics ("putative placebo") AK patients was estimated to be around 19.6% (95%CI: 10.2%; 32.4%), which is far below the clinical resolution rate of Akantior from trial 043 (84.8% with 95%CI: 73.9%; 92.5%). A so-called "study effect" of trial 043 was estimated based on results observed for the chosen comparator in study 043 and Papa et al. 2020 to cover for 30.7% (95%CI: 14.2%; 47.2%) of the observed cure rate. Adding a potential bias of 30.7% results in a resolution for 50.3% (95%CI: 36.6%; 64.1%) of patients without surgery. This still does not overlap with the CIs of clinical resolution rate within 12 months from trial 043.

The difference in proportions in CRR at 12 months between Akantior from study 043 and patients not treated with antiamoebics cases from the systematic literature review is 65.2% (with 95% CI: 49.3%, 77.5%) and adding a potential bias of 30.7% is 34.5% (with 95% CI: 16.8%, 49.8%).

3.3. Uncertainties and limitations about favourable effects

DP batches used for clinical studies (DS from Supplier X) have identified analytical differences to DP batches intended for commercial use (DS from Supplier Y). Even though *in vitro* data did not identify crucial differences between Supplier Y DS and new Supplier X batches (i.e. not used in clinical studies), no data from *in vivo* studies are available. Furthermore, *in vitro* studies were conducted with "new" Supplier X batches and no data at all are available supporting similarity of "old" and "new" Supplier X batches. No clinical data are currently available with the intended commercial product.

In the main study, the monotherapy treatment 0.8 mg/ml polihexanide + placebo failed to show superiority compared to the combination therapy 0.2 mg/ml polihexanide + 1 mg/ml propamidine and the primary objective of the trial was therefore switched from showing superiority to testing non-inferiority. Estimating the difference between the unlicensed reference and placebo in the intended patient population is not possible due to the lack of randomised placebo-controlled trials. Thus, only superiority of the test product to the active comparator would have been interpretable.

The pre-defined 20% NI margin was not adequately justified. There is no sound statistical derivation of the non-inferiority margin to ensure superiority to patients not treated with antiamoebics ("putative placebo"). No clinical justification of the non-inferiority margin is provided. The consequences of untreated *Acanthamoeba* keratitis, potentially resulting in visual loss or even enucleation, make it difficult to define an acceptable non-inferiority margin from a clinical point of view. Due to respective circumstances (no RCT data of the reference to placebo is available and superiority could not be established), the only option was an indirect comparison of the treatment effect of Akantior to patients not treated with antiamoebics.

The estimated resolution rate of patients not treated with antiamoebics (19.6% with 95%CI: 10.2%; 32.4%) is based on a systematic literature review of case reports and case series from 1970-1995 with unclear constancy regarding the primary measure (i.e. resolution). The GRADE assessment was 'low' for the overall certainty of evidence, as inherent bias, such as publication reporting bias, cannot be excluded.

The cure rate of the combination treatment is inconsistent across the retrospective study 038/SI (CRR of 67%), the expanded historical study as published by Papa et al. 2020 (CRR of 53.5%) and actual study results from trial 043/SI (88.5%). The external control threshold (50.3% with 95%CI: 36.6%; 64.1%) is constructed from historically reported resolution rates (19.6% with 95%CI: 10.2%; 32.4%) and the estimated bias (30.7% with 95%CI: 14.2%; 47.2%) between observational studies and trial 043/SI, both of which do not constitute very robust estimations.

3.4. Unfavourable effects

No severe AEs and no SAEs, including severe life-threatening or blinding events, occurred in healthy volunteers.

In study 042, 53 of 90 subjects reported TEAEs during the study. No significant differences in the frequency of TEAEs in the different dosing treatment groups compared to placebo were detected (38.5%, 78.6%, 59.3%, 55.6% in the 0.4 mg/mL, 0.6 mg/ml, 0.8 mg/mL polihexanide dose groups and placebo respectively).

The majority of all TEAEs occurred within the SOCs Eye disorders (30.0% of all subjects), General disorders and administration site condition (21.1% of all subjects) and Investigations (21.1% of all subjects). In all treatment groups, most TEAEs were of mild intensity (85.5%).

There is clear dose-dependent increase in the reporting frequency for TEAEs conjunctival staining and corneal staining (SOC 'Investigations; 7.7%, 21.4% and 40.7% of all subjects in the 0.4 mg/mL, 0.6 mg/ml, 0.8 mg/mL polihexanide group, respectively). Both such TEAEs were not present in the group treated with placebo.

In patients with *Acanthamoeba* keratitis (Phase III study)**,** the overall safety profile of 0.8 mg/ml polihexanide + placebo was similar to the safety profile of the active comparator combination treatment 0.2 mg/ml polihexanide + 1 mg/ml propamidine.

A total of 152 AEs were reported in 60 of 134 subjects. The proportion of subjects with any AE was similar in both treatment groups (44.9% and 44.6% in 0.8 mg/ml polihexanide + placebo arm and active comparator arm respectively).

The majority of reported AEs was mild or moderate in severity. No AEs were assessed as related to the study treatment. Possibly or probably related AEs occurred in 30.4% of patients treated with 0.8 mg/mL polihexanide + placebo and 21.5% of patients treated with 0.2 mg/mL polihexanide + 1.0 mg/mL propamidine.

No deaths occurred in the study. 13 AEs in 9 patients were classified as severe. Severe AEs were evenly distributed between both treatment arms: 7 occurred in 4 patients (5.8%) treated with 0.8 mg/mL polihexanide + placebo and 6 occurred in 5 patients (7.7%) treated with 0.2 mg/mL polihexanide + 1.0 mg/mL propamidine. PTs related to these events were eye pain, corneal perforation (2x), corneal damage resulting in cornea transplant, condition aggravated, scleritis, bacterial keratitis, persistent epithelial defect, hypopyon and deteriorating vision. Four of the 13 reported severe events were further classified as serious according to CIOMS criteria: corneal perforation (1 case in the 0.8 mg/mL polihexanide + placebo arm, 1 case in the combination therapy arm), corneal transplant (1 case in the 0.8 mg/mL polihexanide + placebo arm), and visual impairment (1 case in the 0.8 mg/mL polihexanide + placebo arm).

There were only few isolated non-eye-disorder-related (systemic) AEs in both, healthy volunteers (e.g. headache 10 events) and *Acanthamoeba* keratitis patients (e.g. Nausea 3 events, Dizziness 1 event, headache 1 event).

In the 0.8 mg/mL polihexanide + placebo arm two corneal transplantation and one corneal perforation event occurred in two AK patients. In the comparator arm only one patient experienced a corneal transplantation event. Considering the rather low number of patients in the study, the frequency of having a corneal perforation or transplantation event is three times higher in the 0.8 mg/mL polihexanide monotherapy arm than in the comparator arm.

Three/69 patients in the 0.8 mg/ml polihexanide + placebo arm and 4/65 patients in the 0.2 mg/ml polihexanide + 1 mg/ml propamidine arm had at least one increased IOP measurement (>21 mmHg). Two events of persistent corneal epithelial defect appeared in one patient in the 0.2 mg/ml polihexanide + 1 mg/ml propamidine treatment group. It is not possible to determine whether these AEs were related to polihexanide or propamidine. Concurrent eye disorders were more balanced across treatment groups, but slightly more cases of PT eye pain were reported in the 0.8 mg/ml polihexanide monotherapy group.

3.5. Uncertainties and limitations about unfavourable effects

No ocular or systemic exposure studies have been conducted on non-clinical or clinical level (feasibility on non-clinical level appears given using radiolabelled polihexanide in an appropriate *in vivo* model).

In non-clinical studies polihexanide was found to be a skin sensitizer of moderate to even strong severity. A discussion on the relevance of these findings for the topical ocular administration of polihexanide to *Acanthamoeba* keratitis patients is missing.

The number of patients included in the safety database of 0.8 mg/ml polihexanide is not very high in absolute numbers (27 healthy volunteers, 69 *Acanthamoeba* keratitis patients) hence; uncommon or rare AEs were not detected with a high probability.

There is no clear dose-response relationship in terms of TEAEs and doses up to 0.8 mg/mL polihexanide.

No clinical studies were carried out in special populations except for pediatric, as adolescents (12 – 18 years) were included in the Phase III study. The information related to the pediatric population is limited. There were only three patients younger than 18 years of age (15, 17, 17 years) enrolled that were exposed to 0.8 mg/ml polihexanide. No patients younger than 14 were enrolled.

Limited data for the population 65 years of age and older is available. Only two patients older than 65 years (66, 73 years) were enrolled and exposed to 0.8 mg/ml polihexanide in the Phase III study.

No studies on drug-drug interactions were submitted on clinical or non-clinical level. It is unclear whether potential local drug interactions (e.g. on physicochemical level) with other ocular medications (especially for chronic applications) might be relevant. Principally, additive and/or synergistic and antagonistic pharmacodynamic drug interactions are conceivable.

3.6. Effects Table

Table 82. Effects Table for Akantior for the treatment of *Acanthamoeba* keratitis.

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
Clinical resolution rate at 12 months	Clinically resolved or unresolved AK	%	0.8 mg/ml PHMB + Placebo FAS: 84.8% PPAS: 87.1%	0.2 mg/ml PHMB + 1 mg/ml propamidine FAS: 88.5% PPAS: 89.5%	Superiority was not met, switch to non-inferiority assessment, non-inferiority margin of 20% currently unjustified, comparator unlicensed with unknown magnitude of effect, uncertainties regarding choice of concentration and dosing regimen (especially for the comparator), imbalance of baseline data	Section on Clinical Efficacy 3.3.4.
Time-to-cure	Kaplan-Meier plot on proportion on healed subjects against time since randomisation ; median time to cure	days	0.8 mg/ml PHMB + Placebo 140 days	0.2 mg/ml PHMB + 1 mg/ml propamidine 114 days	Results indicate better outcome for comparator as the log-rank test for the difference between treatment groups is statistically significant in favour of the control arm (p=0.0442).	
Response from baseline	Corneal scarring, corneal ulceration, pupil test, anterior chamber flare, EQ-5D, VFQ-25	% and score from 0-100 for questionnaires (EQ-5D and VFQ-25)	0.8 mg/ml PHMB + Placebo	0.2 mg/ml PHMB + 1 mg/ml propamidine	mostly statistically significant, but might be biased (e.g. bias due to variability in disease history, assessment bias due to knowledge of treatment, temporal variations of disease, regression to the mean)	

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Clinical resolution rate for "untreated" AK patients	Historically reported case reports summarised in a literature review	%	antibiotics, antifungals, antivirals and corticosteroids 19.6% (95%CI: 10.2%; 32.4%)	none	Low certainty of evidence (GRADE), due to possible bias and unclear constancy regarding the primary measure (i.e. resolution). The estimated study effect of trial 043 (30.7%) concludes a constructed resolution for patients not treated with antiamoebics of 50.3% (95%CI: 36.6%; 64.1%)	
Unfavourable Effects						
Adverse events	Balanced occurrence of AEs, no deaths	occurrence	0.8 mg/ml PHMB + Placebo	0.2 mg/ml PHMB + 1 mg/ml propamidine	Low patient numbers - uncommon or rare AEs were not detected with high probability, very limited information regarding subjects <18 and >65 years	Section on Clinical Safety 3.3.7.

Abbreviations: AE: Adverse event, AK: *Acanthamoeba* keratitis, FAS: Full analysis set, PHMB: polihexanide, PPAS: Per protocol analysis set, SAE: Serious adverse event

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

There is no licensed treatment and no standard treatment protocol in the EU/EEA available to treat AK. Thus, patients and ophthalmologists use unlicensed or off-label treatment options. A uniform treatment option throughout the EU with confirmed treatment effect (efficacy and safety) appears beneficial over currently available options. Diamidines and biguanides are considered effective cysticidal anti-amoebic agents. The current approach for the treatment for *Acanthamoeba* keratitis is only possible with off-label therapies and includes biguanides (e.g. polihexanide or chlorhexidine) with or without the addition of diamidines (e.g. propamidine or hexamidine). Thus, the strategy to investigate one of these treatment options in more detail to gain further information regarding the magnitude of treatment effects is acknowledged. A high concentration of polihexanide (Akantior) was tested in a single pivotal phase 3 study as eye drop solution for the treatment of AK. Based on evidence from literature as well as the clinical retrospective study, polihexanide appears to be a suitable treatment option for AK, even though other treatment options (namely the active comparator applied in study 043) appeared to be at least as good.

Considering the choice of the comparator for the pivotal 043 clinical study (unlicensed with unclear magnitude of efficacy and without reasoning regarding the applied dosing scheme) the conclusions based on non-inferiority in this trial are very vague. Furthermore, the justification of the non-inferiority margin (i.e. 0.2) cannot be followed, neither on clinical, nor on statistical grounds. No historical data of the (unlicensed) reference treatment compared to placebo is available and no indirect superiority (of

the study drug over placebo) can therefore be established using this control arm. In conclusion, it is not considered possible to infer efficacy for the treatment based on non-inferiority against the chosen active comparator. Superiority to unapproved treatment options with comparable/same mechanism of action appears difficult to be established in a well-managed CT setting and it is unethical to randomise to placebo. Thus, an indirect comparison to historical reports of "untreated" AK patients is considered a valid strategy to establish a treatment benefit.

Consequently, the pivotal 043 trial is regarded as a single arm trial (rather than a RCT as it was initially planned for) that is relying on external control data to establish an absolute benefit-risk. The external control group of patients not treated with antiamoebics was identified via systematic screening of available literature regarding cases of patients not treated with antiamoebics and was summarised in a systematic literature review (SLR). The approach to establish an absolute treatment benefit via comparison of the Akantior arm in study 043 to historical control data, as for a single arm trial (SAT), is acknowledged. Notably, the possibility of substantial bias (e.g. based on lack of randomization and blinding as well as unclear comparability of the study population to the external population) is inherent in this strategy. Therefore, a rather conservative approach was followed to estimate the treatment effect, still concluding a beneficial treatment effect for Akantior.

The external control threshold for the resolution rate (50.3% with 95%CI: 36.6%; 64.1%) without surgery was estimated from historically reported resolution rates in cases of patients not treated with antiamoebics (19.6% with 95%CI: 10.2%; 32.4%; concluded from the SLR) and the addition of a potential bias/study effect (30.7% with 95%CI: 14.2%; 47.2%), which was concluded from the difference in effect of the comparator treatment as observed between observational studies (Papa et al. 2020) and trial 043/SI. It is acknowledged that the concluded CI of the external control threshold does not overlap with the CIs from trial 043 (clinical resolution rate at 12 months for Akantior: 84.8% with 95%CI: 73.9%; 92.5%). The difference in proportions in CRR at 12 months between Akantior from study 043 and patients not treated with antiamoebics cases reported in the SLR without the estimated study effect is 65.2% (with 95%CI: 49.3%, 92.5%) and considering the study effect is 34.5% (with 95%CI: 16.8%, 49.8%). However, the estimation of resolution rates without treatment as well as the study effect do not constitute very robust estimations. Nevertheless, even under the conservative assumption of 50.3% clinical resolution rate of patients not treated with antiamoebics, the difference to the Akantior treatment effect is sufficiently large and therefore it can be concluded that the treatment with Akantior is beneficial over not treating AK patients.

Ocular administration of polihexanide did not indicate any systemic safety events of concern and clinical safety appears to be comparable between the applied combination treatment and the higher dose of polihexanide. Of note, in both treatment arms the same active substance was part of the treatment regimen and therefore a similar AE reporting is not unexpected. Uncertainties derive from limited patient numbers, especially with respect to the detection of uncommon and rare events. Given the rarity of the disease however, the size of the safety database might finally be acceptable. Also, the limited number of adolescents does not give rise to concern as the infectious disease is the same and adolescent eyes are not anatomically or functionally different from adult eyes in matters of relevance to the disease to be treated. Overall, results from clinical studies do not indicate major risks regarding the ocular use of lower or higher concentrations (up to 0.8 mg/ml applied as single drops, up to 16 times per day) of polihexanide, which is reassuring.

No clinical data were generated with the intended commercial DP and analytical differences were identified between the DP used for clinical trials (DS from Supplier X) and the intended commercial DP (DS from Supplier Y; e.g. molecular weight of the polymers and biguanide content). The lack of *in vivo* data as well as the lack of proven comparability between utilized DP batches leaves uncertainty. However, the intended commercial DP (with DS from Supplier Y) shows more consistent drug product quality as such (compared to the DP based on DS Supplier X) and comparable efficacy and tolerability

was demonstrated for polihexanide with variable quality attributes in the *in vitro* setup. This observation is likely to be attributed to the non-specific mechanism of action, which might not depend on observed variations in biguanide concentration and molecular weight. Also, relevant adverse safety findings at a non-clinical level were only observed with ten times higher concentrations than intended for clinical use, which appears reassuring regarding the expected safety on clinical level with observed variations in quality attributes. The clinical safety profile of Akantior appears rather tolerable, considering the severity of the disease. Furthermore, Akantior is to be used as local application only and systemic exposure appears negligible.

Based on these reassuring considerations, it is concluded that the use of DS from Supplier Y for the commercial DP (instead of the DS from Supplier X employed for clinical studies) does not appear to crucially impact the expected efficacy and safety profile of Akantior.

3.7.2. Balance of benefits and risks

The pivotal 043 study failed to show superiority of Akantior over the active comparator and it remains unclear whether polihexanide alone is indeed non-inferior to the selected active comparator. However, the CHMP considered that there is a clear absolute benefit of treatment with Akantior compared to no treatment (based on a systematic literature review of case reports of AK infections without treatment directed against the underlying infection).

Of note, the DP intended for commercial use was not studied in the clinical trial setup and analytical differences were identified between the DP used for clinical trials (DS from Supplier X) and the intended commercial DP (DS from Supplier Y), which leaves uncertainty regarding the commercial DP. However, based on the reassuring quality of the to-be marketed product Supplier Y, the lack of any consequence of variable quality attributes on *in vitro* measures of efficacy and tolerability, the non-specific mechanism of action that appears independent of e.g. biguanide concentration and molecular weight, the lack of critical safety findings on non-clinical level for doses up to 10-times above the intended clinical level, the clinical tolerability as well as the local application with negligible systemic exposure all are reassuring attributes that allow for the conclusion that the use of the DS from Supplier Y for the commercial DP (instead of the DS from Supplier X employed for clinical studies) does not crucially impact the expected efficacy and safety profile of Akantior.

Additionally, the safety profile of ocular use of the applied high concentration of polihexanide appears manageable considering the devastating disease that is intended to be treated.

Overall, the demonstrated clinical benefits outweigh the risks associated with treatment.

3.7.3. Additional considerations on the benefit-risk balance

CHMP early contact with patient and consumer organisations

In May 2022 the CHMP initiated an early contact with patient and consumer organisations. However, no specific organisation was identified for this disease. One interview was conducted with a single person who had the disease in the recent past. The patient received chlorhexidine 5-times a day and reported that a more frequent treatment regimen would have been tolerable. Both eyes of the patient were affected by the disease, which highlights the need of bilateral disease management for any licensed product. The patient suffered from a delayed diagnosis (1 month after onset) and had to receive a corneal transplant with subsequent development of cataract. It is unclear whether earlier diagnosis might have had a better disease outcome. The shared experience with the disease was noted and is acknowledged. However, the statement of a single person was not considered a sufficiently strong background to refer to in the scope of this report.

Third party intervention during the evaluation of Akantior

During the assessment of this application, the CHMP received correspondences from one ocular diseases association (EuCornea: European Society of Cornea and Ocular Surface Diseases Specialists, hereinafter referred to as "third party") expressing the third party' views about the efficacy profile of Akantior, the unmet medical need of *Acanthamoeba* keratitis the blindness risk of its patients.

The CHMP considered this intervention in the context of its assessment and concluded that the observations put forward by the association were already known by CHMP, and as such had no impact on the CHMP assessment or its conclusions.

3.8. Conclusions

The overall benefit/risk balance of Akantior is positive, subject to the conditions stated in section 'Recommendations'.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Akantior is favourable in the following indication:

treatment of *Acanthamoeba* keratitis in adults and children from 12 years of age.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

-Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription.

-Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

-Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new

information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.