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Safety and tolerability of topical polyhexamethylene biguanide (PHMB): a randomised clinical trial in healthy adult volunteers

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Conflict of interest: Vincenzo Papa and Antonino Asero are employees of SIFI S.p.A. who manufacture and supply PHMB eye drops in Italy, and who are carrying out studies to develop it as a licenced therapy for the treatment of Acanthamoeba keratitis in Europe. John Dart and Darwin Minassian are consultants to SIFI SpA. The remaining authors have no proprietary or commercial interest in any materials discussed in this article.

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Running head: Ocular safety and tolerability of topical PHMB 0.04-0.08%

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Synopsis

PHMB 0.02% is unlicensed but used for treating *Acanthamoeba* keratitis (AK). This study shows that higher concentrations of PHMB (0.04% to 0.08%) are safe in normal eyes justifying their use in AK treatment trials.

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Abstract

Background and aims

Polyhexamethyl biguanide (PHMB), a widely used topical treatment for *Acanthamoeba* keratitis (AK), is unlicensed with no formal safety assessment. This study evaluated its safety and tolerability.

Methods

A prospective randomized double masked controlled trial in 90 healthy volunteers. Subjects were treated with topical 0.04%, 0.06%, 0.08% PHMB or placebo (vehicle) 12x daily for 7 days, then 6x daily for 7 days. The rates of dose limiting adverse events (DLAE) leading to interruption of dosing, mild AE (not dose limiting) and incidental AE (unrelated to treatment), were compared. The primary outcome was the difference between treatments for DLAE rates.

Results

Five of 90 subjects developed DLAE within <1 to 4 days of starting treatment; 2/5 using PHMB 0.06% and 3/5 PHMB 0.08%. These resolved within 1-15 days. There were no significant differences in DLAE between treatment groups. Mild AE occurred in 48/90 subjects (including placebo). There was no trend for an increased incidence of any AE with increasing concentrations of PHMB, except for corneal punctate keratopathy with PHMB 0.08% which fully resolved within 7-14 days.

Conclusion

These findings are reassuring for PHMB 0.02% users. They also suggest that higher PHMB concentrations may show acceptable levels of tolerance and toxicity in AK subjects, whose susceptibility to AE may be greater than for the normal eyes in this study. Given the potential benefits of higher PHMB concentrations for treating deep stromal invasion in AK we think that the use of PHMB 0.08% is justified in treatment trials.

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Introduction

Acanthamoeba keratitis (AK) causes amongst the most prolonged and severe morbidity of any of the causes of acute microbial keratitis [1]. The biguanide antiseptic PHMB, at a concentration of 0.02%, is one of the most widely studied and used topical therapies for AK [2, 3, 4] and recommended by the leading national public health organisation in the USA.[5] However, PHMB is not licensed either as a medicine, or for topical ocular use in the concentrations used in AK, and to date there have been no prospective clinical studies carried out with the primary aim of identifying the toxicity and adverse effects of PHMB.

PHMB was chosen for use at the 0.02% (200 µg/ml) in the first reported case series of 6 AK patients because this concentration exceeded, by approximately 200 fold, the minimum cysticidal concentration (MCC) of 0.97 µg/ml found for the Case 1 clinical isolate and was effective *in vitro* at this concentration against the Case 2 isolate (MCC of 1.90 µg/ml). No PHMB 0.02% toxicity was identified in this study.[6] Subsequently PHMB has shown MCC's of < 4ug/ml in several studies [7, 8], even in persistently culture positive cases [9], establishing PHMB 0.02% as a first line agent in AK. Despite the relative success of PHMB 0.02% (and the alternative biguanide chlorhexidine 0.02%) compared to other drugs for AK failure rates are still in the order of 50% for a poor outcome (acuity less ≤ 20/40 to 20/80 ± surgery), median overall cure times of 5 months[10, 11] and 25% of patients requiring ≥9 months of treatment.[10] As a result PHMB concentrations as high as 0.06% have been recommended [12, 13] for use in clinically resistant AK to increase the penetration of PHMB into the posterior stroma. PHMB 0.02% has poor penetration in a rabbit ex vivo corneal model[14] and failure of drug to penetrate the deep stroma is thought to be a major cause of treatment failure. However, the use of concentrations of PHMB >0.02% has been mentioned in only a handful of cases [10] and any increase in toxicity that might accompany higher concentrations is uncertain.

The aim of this Phase 1 study in healthy human volunteers was to evaluate the adverse effects of concentrations of PHMB 0.04%, 0.06% and 0.08% as part of a program of work designed to develop

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a licensed formulation of topical PHMB, potentially more effective than PHMB 0.02%, for the treatment of AK[15]. The study was done with two aims. First, to provide clinical data on the toxicity and adverse effects of topical PHMB that are needed to satisfy clinicians and patients about its safety. Second, to inform the choice of the highest concentration of PHMB that could be used, without a significant increase in adverse effects, in a current randomised controlled treatment trial.[16]

Methods

The Trial Protocol, with full details of the Trial methodology, is in the Supplementary Appendix; a synopsis of this is provided in the following Methods sections. This was a randomized, double-masked, placebo-controlled, multiple centre, parallel-group Phase I study to evaluate the safety and tolerability of 3 concentrations of preservative-free PHMB 0.04%, 0.06% and 0.08% ophthalmic solution compared to placebo (PHMB vehicle) registered with ClinicalTrials.gov.[17] The study was conducted at 3 clinical study sites in the Netherlands and Belgium, between 13th November 2015 and 17th March 2016, after approval by the local ethics committees (Amsterdam Medical Centre 2015-183#B20 1567 1a, NL54396.018.15; Universitair Ziekenhuis Gent 2015/1182 and Aalst Ref. 2015/103) and complied with Good Clinical Practice and the Declaration of Helsinki.

Subject population, rationale for study numbers, randomization protocol

The study population consisted of healthy volunteers aged 18-54 with no concomitant ocular pathology. The numbers of subjects required for each PHMB treatment group were estimated using binomial probability distribution function which predicted that if no adverse events (AE) were observed in a group of 24 subjects, it could be concluded (with 95% confidence) that the true AE rate is unlikely to be higher than 11.8%. Assuming a dropout rate of 10%, the study aimed to recruit 90 subjects; 27 in each PHMB group and 9 in the placebo group. Subjects were stratified by site and randomly assigned to each of the 3 PHMB treatment groups and the placebo group in a 3:3:3:1 ratio based on a computer-generated randomization schedule using random permuted blocks to ensure that treatment groups were balanced at end of each block. Randomisation was conducted by PCG

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Clinical Services. Supplementary Fig. 1 outlines the subject numbers enrolled, randomised, followed up and analysed.

Treatment and visits

After a screening visit (Visit 1) eligible subjects had a baseline assessment (Visit 2) after which treatment was started. All subjects instilled eye drops 12 times daily (1 drop every hour, daytime only) for 7 days after which they were reassessed (Visit 3, day 7 of treatment) and the treatment frequency reduced to 6 times daily (1 drop every 2 hours, daytime only) for a further 7 days when they were reassessed (Visit 4, day 14 of treatment) following which treatment was terminated. There was a last assessment 7 days after treatment termination (Visit 5, day 21). Subjects with adverse events were reviewed until complete resolution.

Primary and secondary outcomes, assessments and definitions of adverse event categories,.

The primary outcome measure was the difference between treatments for dose limiting adverse event (DLAE) rates and the secondary outcome measure the difference between treatments for mild adverse event (AE) rates. Safety and tolerability assessments consisted of adverse event (AE) reporting, clinical laboratory tests, vital signs and the ocular safety evaluation, carried out by ophthalmologists using a standardised form. The latter included best corrected visual acuity (BCVA), slit-lamp examination, vital staining of the ocular surface with fluorescein and lissamine green, ophthalmoscopy, measurement of intraocular pressure (IOP), analysis of symptoms of ocular discomfort and assessment of global subjective tolerance using the ocular surface disease index (OSDI) grading scale.[18] Adverse events were all adverse clinical events reported during treatment, but not necessarily related to the treatment, occurring in one or more patients. These include the following categories with their definitions:

- Serious adverse events (SAE); life-threatening and resulting in death or requiring inpatient hospitalization.
- Dose limiting adverse events (DLAE); AE resulting in the need to terminate or interrupt treatment.

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- Mild adverse events (Mild AE); not dose limiting during treatment but classified as treatment related.
- Incidental adverse events (IAE); unrelated to treatment.

Events were coded using the Medical Dictionary for Regulatory Activities (MedDRA) 19.0 guideline https://www.meddra.org/sites/default/files/guidance/file/intguide_19_0_english.pdf.

Statistics

SAS® (Version 9.4), SAS Institute Inc., Cary, NC, USA) was used to populate the data Tables with frequencies, proportions, means, standard deviations and confidence intervals. The Fisher's exact test was used for comparison of categorical data between treatment groups overall, and for pairwise comparisons. For the values of IOP, OSDI, conjunctival and corneal staining score, a mixed-model repeated measures analysis was carried out, with adjustment for visit, treatment by visit interaction, and baseline value, for estimation of the adjusted mean change from baseline, and comparison of each PHMB treatment group with placebo. Further analysis of change from baseline values for these three variables included a one-way ANOVA for overall comparison of the PHMB treatment groups at each visit, and pair-wise comparisons using the Tukey Post-hoc test where the overall comparison p-value was <0.05, The Mantel-Haenszel test of trend was used to assess trends of increasing risk of adverse events with increasing concentrations of PHMB.

Results

Table 1 summarises the allocation groups and similar baseline characteristics for the 4 groups of 90 subjects who were randomized, treated and evaluated. Six subjects discontinued treatment because they developed dose limiting AE within 1-4 days of starting treatment and are described below (n=5); the remaining subject discontinued treatment after 4 days of PHMB 0.08% because of alcohol consumption in excess of protocol limits.

Table 2 describes the rates of adverse events categorized as dose limiting AE, mild AE and incidental AE for each group and the statistical comparisons. No serious AE occurred during the study.

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Supplementary Table 1 lists all the individual AE by MedDRA category for all the subjects developing AE in. It is important to understand that these categories of adverse event, and individual adverse events, are not mutually exclusive resulting in 8 adverse events for the 5 subjects developing dose Limiting AE and 124 adverse events for the 48 subjects developing mild AE (see Supplementary Table 1).

Dose limiting AE: the 5/90 subjects (5.6%) who developed these required discontinuation of treatment. These were all ocular (not systemic) AE. Of the five there were 2/28 (7.1%) in the PHMB 0.06% group (1 allergic conjunctivitis, 1 installation site foreign body sensation and 1 conjunctival irritation) and 3/27 (11.1%) in the PHMB 0.08% group (2 with allergic conjunctivitis, 2 with corneal stain and 1 with installation site dryness). These DLAE developed within <1 to 4 days of starting therapy and resolved within 1-15 days of onset. There were no significant differences for the rates of dose limiting AE between groups ($p = 0.418$).

Mild AE: there were 40 different mild AE (mild enough for subjects to continue treatment) of which the three most common were conjunctival stain (16 reports), corneal stain and instillation site pain (12 reports of each). Mild AE developed in 48 subjects; 10 (38.5%) using PHMB 0.04%, 20 (71.4%) using PHMB 0.06%, 13 (48.1%) using PHMB 0.08% and 5 (55.6%) of those using placebo (Table 2). The 124 individual adverse events in these 48 subjects are listed in Supplementary Table 1.

All treatment related AE: Table 2 describes these for each treatment group together with the statistical analysis. There was no significant difference in the proportions of subjects developing a DLAE between groups. Proportions developing any treatment related AE differed ($p = 0.027$) due to a difference between PHMB 0.04% and PHMB 0.06% ($p = 0.005$). There was no evidence for an increasing risk of an AE with an increasing concentration of PHMB (test of trend $p = 0.1896$) either for all groups including placebo, or when analysis was restricted to the three PHMB groups ($p=0.1330$). Table 3 summarises the incidence rates and analysis for the 9 most common AE (defined as events occurring in ≥5% or more of all subjects) for each treatment group. Analysis was carried out

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to evaluate the risk of occurrence of each of these individual AE with increasing concentrations of PHMB. Significant differences were found only for the rates of conjunctival and corneal staining; findings for both were similar both when placebo was included (p=0.0049 and p=0.0043 respectively) and with analysis restricted to the three PHMB groups (p=0.0197 and p=0.0137 respectively). This trend is the result of the difference between the PHMB 0.08% versus PHMB 0.04% group (p=0.04 and p=0.024 respectively). The mean duration of events was 9 days (range 1-28).

IOP, OSDI: the findings and analysis from baseline throughout the treatment period are described in Supplementary Table 2. IOP was unaffected. However, the OSDI findings differed and are shown graphically in Figure 1a with a summary of the statistical analysis; the OSDI scores increased significantly compared to placebo in the PHMB 0.06% and 0.08% groups at both treatment time points after baseline but by the 7 day post treatment evaluation only PHMB 0.06% was significantly higher than placebo. Comparison of the OSDI scores for the different PHMB concentrations showed no significant differences and no trend for an increasing OSDI score with increasing concentrations of PHMB.

Conjunctival (lissamine green) and corneal (fluorescein) staining: the findings and analysis are shown in Supplementary Table 3 and summarised in Figs 1b and c. For conjunctival staining, although there was increased staining in the PHMB groups compared to placebo, the confidence limits (Supplementary Table 2) were wide and differences with placebo were not statistically significant at any time point. There was no indication of an increasing trend associated with increasing PHMB concentrations at any visit. However, corneal staining was increased compared to placebo after a week of 12x daily applications (Visit 3) for all concentrations of PHMB whereas, at the end of a further week of 6x daily applications (Visit 4), the only apparent difference with placebo was for PHMB 0.08%. By the end of the washout period of a week with no applications (Visit 5) there were no clear differences from placebo. There were statistically significant differences in corneal stain scores of different PHMB concentrations at both the end of treatment (Visit 4) (p=0.0126) due to the difference between PHMB 0.08% versus PHMB 0.04% (p=0.0095) and a week later (Visit 5)

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(p=0.0135) due to a higher corneal staining score for PHMB 0.08% compared to both 0.04% (p=0.0342) and 0.06% (p=0.242). Corneal staining had returned to baseline in all subjects by visit 5 (1 week after discontinuation of treatment) apart from two in whom resolution took 2 weeks.

There were no clinically significant changes in vital signs (blood pressure or heart rate) or laboratory variables occurred during the study (data not shown).

DISCUSSION

Phase I studies of ophthalmic drugs are rarely published for critical evaluation. Phase I data are useful to clinicians having to determine whether or not clinical signs are disease or treatment related and to both patients and clinicians to quantify the level of common adverse events. In the treatment of severe disease adverse effects are acceptable if these are infrequently dose limiting and pose limited risks. For example, a randomised controlled trial of ofloxacin 0.3% versus "conventional therapy" with fortified topical gentamicin 1.5% and cefuroxime 5.0% for bacterial keratitis there was a 5 fold higher risk of toxicity with conventional therapy indicating that the risk-benefit of conventional therapy had been acceptable for many years for a severe disease.[19] Data on PHMB toxicity is available from a number of sources. PHMB acts by disrupting acidic phospholipids in microbial cell membranes, as opposed to the neutral phospholipids in human cell membranes, which probably accounts for its low toxicity.[20] In a review of PHMB including *in vitro*, *ex vivo*, *in vivo* and clinical studies, principally for use as an antiseptic for use in skin/oral wound healing, PHMB had a high antimicrobial efficacy with toxicity assessed as low compared to chlorhexidine.[21] In an *in vitro* ocular model there was increased toxicity for PHMB compared to chlorhexidine [22], a similar finding to that in an *ex vivo* rabbit corneal model although the adverse effect on epithelial barrier function was minimal with both biguanides after 6 hours exposure and less than that of the preservative benzalkonium 0.01%. Clinical studies of toxicity have been limited to the evaluation of secondary outcomes of PHMB 0.02% treatment in the context of the early retrospective clinical case series of AK treated patients. Of 111 patients treated with PHMB + propamidine, 29/111 (26%) developed stinging and superficial punctate keratopathy considered to be due to toxicity; in 16/111 (14%) the symptoms resolved on discontinuation of

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propamidine alone, only 1/111 (0.9%) had to discontinue PHMB 0.02% and in 12/111 (11%) of patients the adverse effects were mild permitting continued treatment.[23] In a randomized controlled trial of 51 eyes treated with PHMB 0.02% versus chlorhexidine (CHX) 0.02% monotherapy a mild stinging sensation and superficial punctate keratopathy were recorded for eight cases (differences between drugs not specified). [24] These studies have suggested that PHMB 0.02% rarely results in treatment limiting ocular surface toxicity or corneal endothelial toxicity (given the absence of reports of corneal decompensation in PHMB treated AK).

However, there has been concern about the potential of biguanides, and chlorhexidine to cause cataract and iris atrophy. Eleven cases of cataract, 7 of whom also developed a fixed dilated pupil, have been reported in 2 case series, treated predominantly with chlorhexidine, of whom 3/11 had also been treated with PHMB [25, 26]. The association with toxicity was circumstantial and the potential for the concurrent severe inflammation, topical steroid use or an immune mediated pathology acknowledged. Uncommon and severe side effects like these cannot be identified in Phase I or subsequent Phase studies because of their rarity; their identification is dependent on consistent case reporting.

This human Phase I study evaluated the effect of 2 weeks of intensive topical PHMB 0.04%, 0.06% and 0.08% therapy to replicate the frequency of therapy to be used in the current randomised controlled clinical trial. It was carried out following the results of studies both in cell cultures and in rabbits. The 26 week rabbit study using PHMB 0.08% showed no significant or irreversible ocular surface AE and no intraocular AE after 16x daily applications, from day 1-5, followed by 8x daily applications from day 6-21 and then 4x daily applications from weeks 4-26.[27] In the present study, only 5/90 subjects developed dose limiting AE resulting in the need to withdraw treatment; these all developed within <1-4 days of starting treatment with 0.06% PHMB (2 subjects) and 0.08% PHMB (3 subjects) and resolved within 1-15 days of onset. Mild AE occurred in 48/90 subjects (including 10 AE in 5 of 9 subjects using placebo) and resolved within 1-28 days of onset. Differences between groups for the development of any AE were shown for PHMB 0.04% versus 0.06% but there was no

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trend for an increased incidence with increasing concentrations of PHMB (Table 2). For the 9 most common AE (Table 3) there was increased incidence only for conjunctival and corneal vital stain which was due to the increased incidence of both in those using PHMB 0.08% versus those using PHMB 0.04%. There is evidence of mild ocular surface damage with the intensive use of PHMB 0.08% which resolved within a week in all but 2 subjects in whom this took 2 weeks.

The principal limitation of this study is that it was carried out in normal individuals without the ocular inflammation or epithelial defects that are present in *Acanthamoeba* keratitis. Although these findings cannot fully predict whether there might be additional toxicity in keratitis patients, they suggest both that the risk of having to discontinue therapy using concentrations of PHMB up to 0.08% will probably be low, and also that adverse events are likely to be few. Given the potential benefits of higher concentrations of PHMB for advanced AK affecting the deep corneal stroma, we think that their use is justified in a Phase III trial. As a result PHMB 0.08% monotherapy is being employed in a current Phase III randomised controlled treatment trial for AK[16] for which recruitment is now complete. Nevertheless, until the adverse event reports from this trial have been analysed, we will not know whether there may be additional toxicity findings using this higher concentration of PHMB in keratitis patients. However, for the currently widely used concentration of 0.02%, we hope that these data will be reassuring to clinicians when recommending, and to patients when using, this currently unlicensed drug.

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Legend for Figure 1

The plots show the changes in scores with the baseline fixed at zero. The full data and analyses summarised here are in Supplementary Tables 2 and 3. Timepoints are Baseline - before treatment; Visit 3 - after 7 days of 12x daily treatment application; Visit 4 - after a subsequent 7 days of 6x daily application; and Visit 5 - after a further 7 days with no applications.

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Table 1
Demographic data

	Treatment groups				
Characteristics	**PHMB 0.04% (n=26)**	**PHMB 0.06% (n=28)**	**PHMB 0.08% (n=27)**	**Placebo (n=9)**	**Total (n=90)**
Age (years)					
Mean (Standard deviation SD)	30.5 (12.5)	29.4 (8.7)	30.4 (10.3)	25.7 (4.6)	29.6 (10.1)
Minimum (Min), Maximum (Max)	18,54	20,49	18,53	18,32	18,54
Weight (kg)					
Mean (SD)	71.2 (14.6)	73.5 (11.6)	76.8 (9.9)	68.3 (15.1)	73.3 (12.5)
Min, Max	51.6,109.0	56.0,94.2	55.4,102.5	54.0,103.0	51.6,109.0
BMI (kg/m^2)					
Mean (SD)	23.2 (2.8)	23.6 (2.5)	24.2 (2.7)	23.3 (3.2)	23.6 (2.7)
Min, Max	20,29	20,28	20,29	21,29	20,29
Gender					
Male	12 (46.2%)	13 (46.4%)	17 (63.0%)	2 (22.2%)	44 (48.9%)
Female	14 (53.8%)	15 (53.6%)	10 (37.0%)	7 (77.8%)	46 (51.1%)

There were no statistically significant differences between the proportions of males and females in each group (overall 2-sided Fisher's exact test p = 0.190)
The higher proportion of males in the PHMB 0.08% group compared to Placebo was not significantly different at the 5% level (Fisher's exact test p=0.055)

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Table 2
Overall summary of safety described by the rates of patients developing adverse events in different treatment groups

	Number (n) and percentage (%) of adverse events for all treatment groups				
Adverse event category[a]	**PHMB 0.04% (n =26) n (%)**	**PHMB 0.06% (n=28) n (%)**	**PHMB 0.08% (n=27) n (%)**	**Placebo (n=9) n (%)**	**Total (n=90) n (%)**
Serious adverse events (SAE)[b]	0	0	0	0	0
Dose limiting adverse events (DLAE)[c] *	0	2 (7.1%)	3 (11.1%)	0	5 (5.6%)
Non dose limiting adverse events (Mild AE)[d]	10 (38.5%)	20 (71.4%)	13 (48.1%)	5 (55.6%)	48 (53.3%)
All treatment related AE (total of DLAE and Mild AE)**†	10 (38.5%)**	22 (78.6%)**	16 (59.3%)	5 (55.6%)	53 (58.9%)
Incidental adverse events (IAE)[e] (unrelated to therapy)	1	1	0	0	2
Total number of adverse events (AE)[f] n	11 (42.3%)	23 (82.1%)	16 (59.3%)	5 (55.6%)	55 (61.1%)

Legends:

a. Individual adverse events, in each category, are listed in Supplementary Table 1.
b. SAE are life-threatening and resulting in death or requiring inpatient hospitalization.
c. DLAE are defined as those AE resulting in termination or interruption of treatment. These occurred in 5 subjects and were allergic conjunctivitis, corneal staining, ocular dryness, foreign body sensation and conjunctival irritation of mild to moderate intensity. All resolved within 1-15 days of onset.
d. Mild AE are adverse events occurring during treatment, which did not prevent continuation of treatment, and which were classified as related to the treatment.
e. Incidental AE are adverse events are those thought to be unrelated to treatment.
f. AE are defined as any adverse clinical events reported during treatment, but not necessarily related to the treatment, occurring in one or more patients and for which the number of affected patients is reported.

Statistical analysis

* There was no statistically significant difference between the proportions of subjects developing DLAE in the different groups (overall 2-sided Fisher's exact test p = 0.418).

** The proportion of subjects developing DLAE and Mild AE differs between groups (overall 2-sided Fisher's exact test p = 0.027) which pairwise comparison shows is due to the difference between PHMB 0.04% and PHMB 0.06%. (Fisher's exact p = 0.005).

† There was no statistically significant trend of increasing risk of the development of TEAE with increasing concentrations of PHMB (Maentel-Haenszel test of trend p = 0.1896, test for homogeneity p = 0.0306) either for all groups, including placebo, or for analysis restricted to the three PHMB groups (p=0.1330, test of homogeneity 0.0118).

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Table 3
Comparison of the rates of the most common treatment related adverse events for all treatment groups

	Number (n) of common adverse events for all Trial subsets				
Common adverse events (reported in ≥ 5% of subjects)	**PHMB 0.04% (n=26) n**	**PHMB 0.06% (n=28) n**	**PHMB 0.08% (n=27) n (%)**	**Placebo (n=9) n (%)**	**Total (n=90) n (percent)**
1. Conjunctival staining*	2†	5	9†	0	16 (17.8)
2. Corneal staining+	1#	5	8#	0	14 (15.6)
3. Instillation site pain	2	4	5	1	12 (13.3)
4. Conjunctival hyperemia	1	4	4	0	9 (10.0)
5. Headache	3	4	1	1	9 (10.0)
6. Eyelid edema	0	6	0	0	6 (6.7)
7. Instillation site erythema	2	3	0	0	5 (5.6)
8. Instillation site foreign body sensation	1	2	1	1	5 (5.6)
9. Instillation site pruritus	1	3	1	0	5 (5.6)

Footnotes:

a. Coded using the Medical Dictionary for Regulatory Activities (MedDRA) 19.0 terms
b. A complete list of all TEAE is included in the online Appendix

Statistical analysis

Analysis was carried out to evaluate the risk of occurrence of each individual AE with increasing concentrations of PHMB. Statistically significant differences were found only for conjunctival and corneal surface staining and not for the remaining 7 events.

* For conjunctival staining there was a statistically significant increase in the incidence with increasing concentrations of PHMB (Maentel-Haenszel test of trend p = 0.0049, test of homogeneity 0.0433) which was similar when the analysis was restricted to the 3 PHMB groups (p = 0.0197).

† Pair-wise comparisons identified only one significant difference for PHMB 0.04 vs 0.08% (Fisher's exact p = 0.039).

+ For corneal staining results were very similar using the same methods showing an increasing trend in the incidence for increasing concentrations of PHMB (p = 0.0043, test of homogeneity 0.0374) that was similar when the analysis was restricted to the 3 PHMB groups (p = 0.0137)

Pair-wise comparisons identified a significant difference for PHMB 0.04% vs 0.08% significance finding (p = 0.024).

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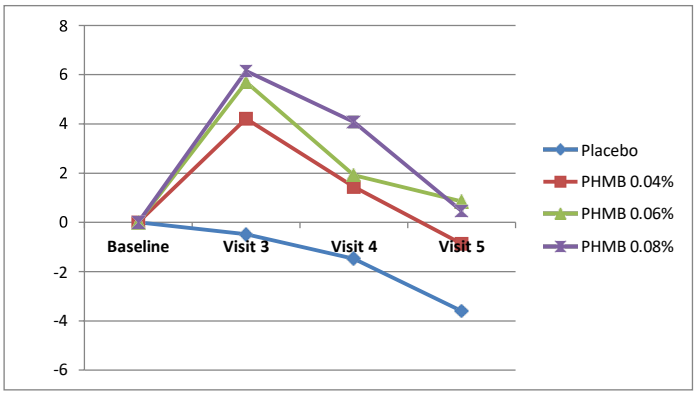
Figure 1
Changes in scores for the Ocular Surface disease Index (OSDI), conjunctival and corneal staining with a summary of the statistical analysis

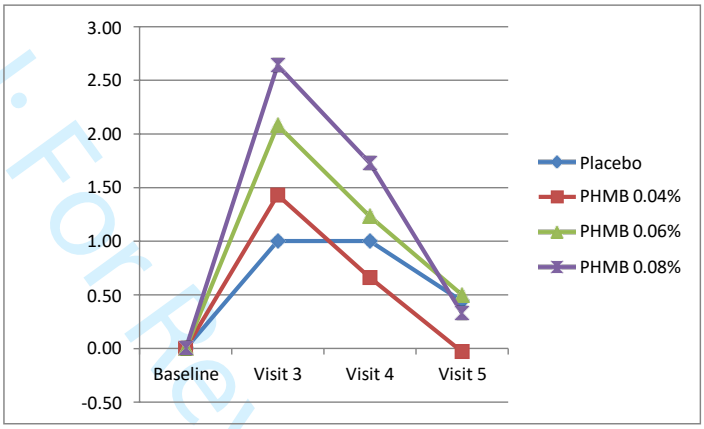
Ia OSDI

The OSDI scores increased, at statistically significant levels by Fisher's exact test (Fisher's) compared to placebo, in the 0.06% PHMB and 0.08% PHMB groups at 2 time points after Baseline. By the 7 day post-treatment evaluation (Visit 5) the only significant difference was for PHMB 0.04% vs placebo. No significant differences were shown in the OSDI for any of the PHMB concentrations at any visit using either one-way analysis of variance (ANOVA) or with pairwise comparisons using the Tukey HSD post-hoc test (Tukey).



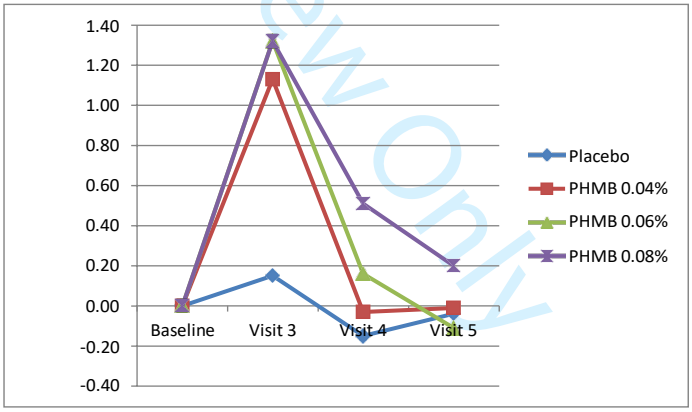
Ib Conjunctival staining (lissamine green)

Although there was increased staining in the PHMB groups compared to placebo the confidence limits were wide and differences with placebo were not statistically significant at any time point (Fisher's). No significant differences between different PHMB concentrations were shown for any visit using either ANOVA or pairwise comparisons (Tukey).



1c Corneal staining (fluorescein)

Corneal staining showed significant increases compared to placebo after 7 days of 12x daily applications (Visit 3) for all concentrations of PHMB whereas, at the end of a further 7 days of 6x daily applications (Visit 4), the only significant difference with placebo was for PHMB 0.08%. At the end of the 7 day post-treatment period (Visit 5) there were no significant differences from placebo (Fisher's). Significant differences using ANOVA) were shown for corneal staining between the PHMB concentrations at the Visit 4 assessment due to the increased staining for PHMB 0.08% vs PHMB 0.04% (p=0.0095, Tukey) and at the 7 day post-treatment assessment due to increased staining in the PHMB 0.08% group compared to both PHMB 0.04% (p=0.0342) and 0.06% (p=0.242). In all cases staining had returned to baseline within 1-2 weeks of discontinuing treatment.



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Supplementary Table 1
Incidence of Adverse Events
Coded using the Medical Dictionary for Regulatory Activities (MedDRA) 19.0 guideline
Italicised adverse events in the left-hand column below are the nine most common: these are analysed in Table 3.

Incidence of DLAE
Note: some subjects had more than one adverse event resulting in 8 recorded adverse events in the 5 subjects

	DRUG GROUPS				
ADVERSE EVENTS	**PHMB 0.04% (n=26) n (%)**	**PHMB 0.06% (n=28) n (%)**	**PHMB 0.08% (n=27) n (%)**	**Placebo (n=9) n (%)**	**Total (n=90) n (%)**
Subjects with Dose limiting AE (DLAE)	**0**	**2 (7.1%)**	**3 (11.1%)**	**0**	**5 (5.6%)**
List of AE					
Conjunctivitis allergic	-	1	2	-	3
*Vital dye staining cornea present**	-	-	2	-	2*
Instillation site dryness	-	-	1	-	1
*Instillation site FB sensation**	-	1	-	-	1*
Conjunctival irritation	-	1	-	-	1
Total of all DLAE	**-**	**3**	**5**	**-**	**8**

Incidence of Mild AE
Described by **category** (in bold type) and by individual AE within each category.
Note: some subjects had more than one mild AE in more than one **category** (these are not mutually exclusive).
For each **category** numbers (n) and percentages (%) give number of subjects, of those with mild AE, developing an adverse event.
The adverse events are not mutually exclusive, and more than one may have developed in each subject.
The number of each type of adverse event is given for each drug group and a total number for all affected by mild AE

	PHMB 0.04% (n=26)	**PHMB 0.06% (n=28)**	**PHMB 0.08% (n=27)**	**Placebo (n=9)**	**Total (n=90)**
Subjects with Mild (not dose limiting) AE	**10 (38.5%)**	**20 (71.4%)**	**13 (48.1%)**	**5 (55.6%)**	**48 (53.3%)**
Blood & lymphatic disorders subjects affected (%)	**0**	**0**	**0**	**1 (20%)**	**1 (2.1%)**
Anaemia	-	-	-	1	1
Eye disorders subjects affected (%)	**4 (40%)**	**11 (55%)**	**7(53.8%)**	**1 (20%)**	**27 (56.3%)**
Abnormal sensation in eye	-	-	-	1	1
Conjunctival follicles	2	-	-	-	2
*Conjunctival hyperaemia**	1	4	4	-	9*
Conjunctival irritation	-	2	1	-	3
Conjunctival oedema	-	1	-	-	1
Conjunctivitis allergic	-	-	-	-	-
Erythema of eyelid	-	1	-	-	1
Eye discharge	1	3	-	-	4
Eye irritation	-	-	-	1	1
Eye pain	-	1	-	1	2
Eyelid cyst	-	1	-	-	1
*Eyelid oedema**	-	6	-	-	6*
Lacrimation increased	-	-	1	-	1
Ocular discomfort	-	1	-	-	1
Ocular hyperaemia	1	1	-	-	2
Photophobia	-	1	-	-	1
Retinal pigment epitheliopathy	-	-	1	-	1
Vision blurred	-	2	1	-	3
Subtotal of Eye events	5	24	8	3	40
Gastrointestinal (GI) disorders subjects affected	**0**	**0**	**2 (15.4%)**	**0**	**2 (4.2%)**
Dental caries	-	-	1	-	1
Vomiting	-	-	1	-	1
Subtotal of GI events	0	0	2	0	2

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General disorders (GD) & administration site subjects affected	**4 (40%)**	**6 (30%)**	**5 (38.5%)**	**2 (22.2%)**	**19 (39.6%)**
Instillation site dryness	-	1	1	-	2
*Instillation site erythema**	2	3	-	-	5*
*Instillation site foreign body sensation**	1	1	1	1	4*
Instillation site lacrimation	-	1	-	-	1
*Instillation site pain**	2	4	5	1	12*
*Instillation site pruritus**	1	3	1	-	5*
Subtotal of GD events	6	13	8	2	29
Infections and infestations (I&I) subjects affected	**0**	**7 (35%)**	**1 (7.7%)**	**2 (22.2%)**	**10 (20.8%)**
Conjunctivitis	-	1	-	-	1
Influenza	-	1	1	1	3
Nasopharyngitis	-	3	-	1	4
Oral herpes	-	1	-	-	1
Urinary tract infection	-	1	-	-	1
Subtotal of I&I events	0	7	1	2	10
Injury, poisoning and procedural complications	**0**	**1 (5%)**	**0**	**0**	**1 (2.1%)**
Contusion	-	1	-	-	1
Investigations (Inv)	**2 (20%)**	**6 (30%)**	**11 (84.6%)**	**0**	**19 (39.6%)**
*Conjunctival staining**	2	5	9	-	16*
*Vital dye staining cornea present**	1	5	6	-	12*
Subtotal of Inv events	3	10	15	0	28
Musculoskeletal and connective tissue (MS) disorders	**0**	**0**	**1(7.7%)**	**1 (11.1%)**	**2 (4.2%)**
Arthralgia	-	-	1	-	1
Pain in jaw	-	-	-	1	1
Subtotal MS events	0	0	1	1	2
Nervous system disorders	**3 (30%)**	**4 (20%)**	**1 (7.6%)**	**1 (11.1%)**	**9 (18.8%)**
*Headache**	3	4	1	1	9*
Reproductive system and breast disorders	**0**	**1 (5%)**	**0**	**0**	**1 (2.1%)**
Dysmenorrhea	-	1	-	-	1
Respiratory, thoracic and mediastinal disorders	**0**	**0**	**1 (7.6%)**	**0**	**1 (2.1%)**
Epistaxis	0	0	1	0	1
Total of all Mild AE	**17**	**60**	**37**	**10**	**124**

	PHMB 0.04% (n=26)	**PHMB 0.06% (n=28)**	**PHMB 0.08% (n=27)**	**Placebo (n=9)**	**Total (n=90)**
Subjects with incidental adverse events (IAE) of Total number in each drug group	**1**	**1**	**0**	**0**	**2**
Headache	1	-	-	-	1
Influenza	-	1	-	-	1
Subtotal IA events	1	1	0	0	2

* Indicates the nine most common adverse events and the total number of these reported as Dose limiting AE's or Mild AE's

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Supplementary Table 2

Intraocular pressure (IOP) and Ocular Surface Disease Index (OSDI) grades
Arranged for each group by visit with an analysis of trends and differences between different concentrations of PHMB for the OSDI.

Timepoints: Baseline - before treatment; Visit 3 - after 7 days of 12x daily treatment application; Visit 4 - after a subsequent 7 days of 6x daily application; Visit 5 - after a further 7 days with no applications. Numbers in each group vary at each visit.

Parameter	Timepoint	Statistic	Groups			
			0.04%	**0.06%**	**0.08%**	**Placebo**
IOP, (mmHg)	**Baseline**	**Number**	**26**	**28**	**27**	**9**
		Mean (SD)[a]	15.8 (1.8)	16.7 (1.9)	16.0 (2.0)	16.2 (1.4)
	Visit 3	**Number**	**26**	**26**	**22**	**5**
		Mean (SD)	15.7 (1.8)	15.5 (1.5)	15.3 (2.1)	16.1 (2.3)
		Change from baseline (95% CI[b]) P value vs placebo	-0.28 (-0.84, 0.29) 0.753	-0.91 (-1.48, -0.34) 0.152	-0.72 (-1.32, -0.11) 0.282	-0.10 (-1.06, 0.86)
	Visit 4	**Number**	**26**	**26**	**22**	**9**
		Mean (SD)	15.3 (1.8)	14.9 (1.7)	15.2(1.4)	15.3 (2.4)
		Change from baseline (95% CI) P value vs placebo	-0.64 (-1.25, -0.03) 0.745	-1.47 (-2.08, -0.86) 0.304	-0.89 (-1.54, -0.24) 0.939	-0.84 (-1.88, 0.20)
	Visit 5	**Number**	**26**	**28**	**27**	**9**
		Mean (SD)	14.9 (1.9)	15.5 (2.6)	15.6 (1.8)	16.0 (2.9)
		Change from baseline (95% CI) P value vs placebo	-1.03 (-1.80, -0.25) 0.249	-0.96 (-1.70, -0.21) 0.284	-0.50 (-1.26, 0.26) 0.636	-0.14 (-1.45, 1.18)
OSDI, (score)	**Baseline**	**Number**	**26**	**28**	**27**	**9**
		Mean (SD)	3.3 (7.7)	3.7 (3.2)	2.4 (4.2)	5.7 (7.2)
	Visit 3	**Number**	**26**	**26**	**23**	**9**
		Mean (SD)	7.6 (11.7)	8.8 (7.3)	8.5 (7.5)	4.4 (4.6)
		Change from baseline (95% CI) P value vs placebo	4.21 (1.18, 7.23) 0.122	5.70 (2.68, 8.71) **0.043**	6.15 (2.94, 9.35) **0.033**	-0.48 (-5.64, 4.67)
	Visit 4	**Number**	**26**	**26**	**23**	**9**
		Mean (SD)	4.8 (5.8)	5.0 (5.0)	6.4 (7.1)	3.4 (3.8)
		Change from baseline (95% CI) P value vs. placebo	1.44 (-0.37, 3.25) 0.109	1.92 (0.13, 3.72) 0.061	4.08 (2.18, 5.97) **0.003**	-1.48 (-4.58, 1.61)
	Visit 5	**Number**	**26**	**28**	**27**	**9**
		Mean (SD)	2.5 (4.9)	4.5 (8.3)	3.2 (5.6)	1.3 (2.7)
		Change from baseline (95% CI) P value vs. placebo	-0.87 (-3.00,1.26) 0.202	0.85 (-1.20,2.90) **0.037**	0.46 (-1.63, 2.56) 0.059	-3.60 (-7.23,0.04)

Footnotes:
a. SD = standard deviation b. CI = confidence interval
Statistically significant differences (≤ 0.05) for pairwise comparisons with placebo are highlighted in bold.

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Differences between different concentrations of PHMB for OSDI

Differences between PHMB concentrations for clinical findings at each visit were assessed using a one-way ANOVA with an approximate estimate of standard errors from the confidence intervals. The Tukey Post-hoc test was then used for pairwise comparison of the PHMB concentrations at each visit. The results are described below and showed no differences. Pairwise comparisons are not displayed where the ANOVA overall comparison shows no significant difference ($p \geq 0.05$)

One-way Analysis of Variance (ANOVA)

OSDI score: Adjusted mean Change from Baseline				**Overall Between Groups Comparison**		
Visit 3	n	mean change from baseline	se	d.f.	F	p-value
PHMB 0.04%	26	4.21	1.4709			
PHMB 0.06%	26	5.70	1.4660			
PHMB 0.08%	27	6.15	1.5613			
	Overall comparison between groups:			2.00	0.457	0.6352

Visit 4	n	mean change from baseline	se	d.f.	F	p-value
PHMB 0.04%	26	1.44	0.8786			
PHMB 0.06%	26	1.92	0.8732			
PHMB 0.08%	23	4.08	0.9161			
	Overall comparison between groups:			2.00	2.401	0.0978

Visit 5	n	mean change from baseline	se	d.f.	F	p-value
PHMB 0.04%	26	-0.87	1.034			
PHMB 0.06%	28	0.85	1.000			
PHMB 0.08%	27	0.46	1.0195			
	Overall comparison between groups:			2	0.775	0.4642

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Supplementary Table 3

Lissamine green (conjunctival) and fluorescein (corneal) staining grades
Arranged for each group by visit with an analysis of trends and differences between different concentrations of PHMB

Timepoints are: Baseline - before treatment; Visit 3 - after 7 days of 12x daily treatment application; Visit 4 - after a subsequent 7 days of 6x daily application; Visit 5 - after a further 7 days with no applications. Numbers in each group vary at each visit.

Parameter	Timepoint	Statistic	Groups			
			0.04%	**0.06%**	**0.08%**	**Placebo**
Conjunctival staining	**Baseline**	**Numbers**	**26**	**28**	**27**	**9**
		Mean (SD)[a]	0.5 (1.0)	0.4 (0.8)	0.3 (0.7)	0.3 (0.5)
	Visit 3	**Numbers**	**26**	**26**	**23**	**9**
		Mean (SD)	1.9 (2.3)	2.3 (2.5)	2.9 (2.6)	1.3 (1.6)
		Change from baseline (95% CI[b]) P value vs placebo	1.43 (0.59, 2.28) 0.607	2.08 (1.24, 2.92) 0.200	2.64 (1.74,3.53) 0.057	1.00 (-0.44, 2.43)
	Visit 4	**Numbers**	**26**	**26**	**22**	**9**
		Mean (SD)	1.1 (1.6)	1.5 (2.0)	2.1 (2.1)	1.3 (1.7)
		Change from baseline (95% CI) P value vs placebo	0.66 (0.01, 1,31) 0.604	1.23 (0.58, 1.88) 0.716	1.73 (1.02, 2.43) 0.273	1.00
	Visit 5	**Numbers**	**26**	**28**	**27**	**9**
		Mean (SD)	0.4 (0.9)	0.9 (2.0)	0.6 (0.9)	0.8 (1.0)
		Change from baseline (95% CI) P value vs placebo	-0.03 (0.05. 0.44) 0.316	0.50 (0.04, 0.95) 0.912	0.33 (-0.14, 0.79) 0.807	0.44 (-0.36,1.24)
Corneal staining	**Baseline**	**Numbers**	**26**	**28**	**27**	**9**
		Mean (SD)	0.2 (0.4)	0.2 (0.5)	0 (0.2)	0.2 (0.7
	Visit 3	**Numbers**	**26**	**26**	**23**	**9**
		Mean (SD)	1.3 (1.8)	1.5 (1.8)	1.3 (1.4)	0(0)
		Change from baseline (95% CI) P value vs placebo	1.13 (0.49, 1.77) **0.047**	1.32 (0.68, 1.95) **0.023**	1.32 (0.65, 2.00) **0.025**	-0.15 (-1.23,0.94)
	Visit 4	**Numbers**	**26**	**26**	**22**	**9**
		Mean (SD)	0.1 (0.4)	0.3 (0.7)	0.5 (0.7)	0 (0)
		Change from baseline (95% CI) P value vs. placebo	-0.03 (-0.28,0.23) 0.636	0.16 (-0.09,0.41) 0.227	0.51 (0.24,0.78) **0.013**	-0.15 (-0.58,0.29)
	Visit 5	**Numbers**	**26**	**28**	**27**	**9**
		Mean (SD)	0 (0.2)	0 (0.2)	0.3 (0.7)	0.1 (0.3)
		Change from baseline (95% CI) P value vs. placebo	-0.10 (-0.28, 0.07) 0.697	-0.11 (-0.27,0.06) 0.674	0.20 (0.03,0.37) 0.172	-0.04 (-0.33,0.26)

Footnote: a. SD = standard deviation b. CI = confidence interval
Statistically significant differences (≤ 0.05) for pairwise comparisons with placebo are highlighted in bold.

Differences between different concentrations of PHMB for conjunctival and corneal staining
Differences between PHMB concentrations for these clinical findings at each visit were assessed using a one-way ANOVA with an approximate estimate of standard errors from the confidence intervals. The Tukey Post-

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hoc test was then used for pairwise comparison of the PHMB concentrations at each visit. The results are shown below. Pairwise comparisons are not shown where the ANOVA overall comparison shows no significant difference (p>0.05)

Conjunctival stain

One-way Analysis of Variance (ANOVA)

Conjunctival Staining score: Adjusted mean Change from Baseline				**Overall Between Groups Comparison**		
Visit 3	n	mean change from baseline	se	d.f.	F	p-value
PHMB 0.04%	26	1.43	0.407767			
PHMB 0.06%	26	2.08	0.407767			
PHMB 0.08%	23	2.64	0.433944			
	Overall comparison between groups:			2.00	2.0786	0.1325

Visit 4	n	mean change from baseline	se	d.f.	F	p-value
PHMB 0.04%	26	0.66	0.407767			
PHMB 0.06%	26	1.23	0.407767			
PHMB 0.08%	22	1.73	0.433944			
	Overall comparison between groups:			2	2.6544	0.0773

Visit 5	n	mean change from baseline	se	d.f.	F	p-value
PHMB 0.04%	26	-0.03	-0.03883			
PHMB 0.06%	28	0.5	0.224390			
PHMB 0.08%	27	0.33	0.229268			
	Overall comparison between groups:			2	2.0118	0.1406

Corneal stain

One-way Analysis of Variance (ANOVA)

Corneal Staining score: Adjusted mean Change from Baseline				**Overall Between Groups Comparison**		
Visit 3	n	mean change from baseline	se	d.f.	F	p-value
PHMB 0.04%	26	1.13	0.310680			
PHMB 0.06%	26	1.32	0.310680			
PHMB 0.08%	23	1.32	0.323047			
	Overall comparison between groups:			2	0.1238	0.8837

Visit 4	n	mean change from baseline	se	d.f.	F	p-value
PHMB 0.04%	26	-0.03	0.121359			
PHMB 0.06%	26	0.16	0.121359			
PHMB 0.08%	22	0.51	0.129808			
	Overall comparison between groups:			2	4.654	**0.0126**

Pair-wise comparisons: Tukey HSD Post-hoc Test

PHMB 0.04% vs PHMB 0.06%: Diff=0.1900, 95%CI=-0.2189 to 0.5989, p=0.5097

PHMB 0.04% vs PHMB 0.08%: Diff=0.5400, 95%CI=0.1129 to 0.9671, p=0.0095

PHMB 0.06% vs PHMB 0.08%: Diff=0.3500, 95%CI=-0.0771 to 0.7771, p=0.1293

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Visit 5	n	mean change from baseline	se	d.f.	F	p-value
PHMB 0.04%	26	-0.10	0.087379			
PHMB 0.06%	28	-0.11	0.078049			
PHMB 0.08%	27	0.20	0.082685			
	Overall comparison between groups:			2	4.5544	**0.0135**
Pair-wise comparisons: Tukey HSD Post-hoc Test						
PHMB 0.04% vs PHMB 0.06%: Diff=-0.0100, 95%CI=-0.2893 to 0.2693, p=0.9949						
PHMB 0.04% vs PHMB 0.08%: Diff=0.3000, 95%CI=0.0182 to 0.5818, p=0.0342						
PHMB 0.06% vs PHMB 0.08%: Diff=0.3100, 95%CI=0.0334 to 0.5866, p=0.0242						

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Supplementary Appendix

Phase I Trial Protocol

The European Orphan Drug for Acanthamoeba Keratitis (ODAK) Consortium

Randomized, Double-Masked, Placebo-Controlled Multiple-Dose Phase 1 Study to Evaluate the Safety and Tolerability of Different Doses of Preservative-free Polyhexamethylene Biguanide (PHMB) Ophthalmic Solution in Healthy Subjects

Protocol Number: ODAK Phase I (042/SI)

Sponsor	SIFI SpA Via Ercole Patti, 36 95025 Lavinaio - Aci S. Antonio (CT) Italy
Contract Research Organization	PSR Crown Business Center Schiphol Planetenweg 5 2132 HN Hoofddorp The Netherlands
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DATE:	Amendment 2, 04 December 2015
EudraCT Number:	2015-002979-15

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Signature Dec 04,2015 Date

Signature 2015-12-04 Date

GCP Compliance

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

Confidentiality Statement

The information in this document contains trade secrets and commercial information that are privileged or confidential and may not be disclosed unless such disclosure is required by applicable law or regulations. In any event, persons to whom the information is disclosed must be informed that the information is privileged or confidential and may not be further disclosed by them. These restrictions on disclosure will apply equally to all future information supplied to you that is indicated as privileged or confidential.

INVESTIGATOR STATEMENT

I have read the protocol entitled "Randomized, double-masked, placebo-controlled multiple-dose Phase 1 study to evaluate the safety and tolerability of different doses of preservative-free polyhexamethylene biguanide (PHMB) ophthalmic solution in healthy subject" and the accompanying Investigator's Brochure. I agree to conduct the clinical investigation in compliance with the Final Protocol, Amendment 2 Version, dated 04 December 2015, the International Conference on Harmonisation (ICH) harmonised tripartite guideline E6(R1): Guideline for Good Clinical Practice, applicable regulatory/government regulations, and in accordance with the latest revision of the Ethical Principles for Medical Research Involving Human Subjects (the Declaration of Helsinki). I will not implement any changes to study procedures or conduct without prior approval from the ODAK consortium and, when applicable, the Independent Ethics Committee and Regulatory Authority.

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

Signature of Principal Investigator

Date

Printed Name of Principal Investigator

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SYNOPSIS

Study Title: Randomized, Double-Masked, Placebo-Controlled Multiple-Dose Phase 1 Study to Evaluate the Safety and Tolerability of Different Doses of Preservative-free Polyhexamethylene Biguanide (PHMB) Ophthalmic Solution in Healthy Subjects

OBJECTIVES

The primary objective of the study is to establish the ocular safety and tolerability, and systemic safety of 3 different concentrations of preservative-free PHMB in healthy subjects. Safety and tolerability will be compared to those of a placebo.

The PHMB bioavailability in plasma will also be assessed.

HYPOTHESIS

No formal hypothesis testing is planned.

OVERVIEW OF STUDY DESIGN

This is a randomized, double-masked, placebo-controlled, multiple center, parallel-group Phase 1 study to evaluate the safety and tolerability of 3 doses of preservative-free PHMB ophthalmic solution compared to placebo in healthy male and female subjects. The study consists of an eligibility screening visit, 1 treatment period including short ambulant visits, and a follow-up visit.

In total 90 subjects will be assigned to one of the following 4 treatment groups in a ratio of 3:3:3:1:

Group 1: 0.04% PHMB, n=27

Group 2: 0.06% PHMB, n=27

Group 3: 0.08% PHMB, n=27

Group 4: placebo, n=9

In each group, subjects will receive the study drug/placebo 12 times daily (1 drop every hour, daytime only) for 7 days (Days 0-6) and, if well tolerated, followed by 6 times daily (1 drop every 2 hours, daytime only) for an additional 7 days (Days 7 to 13). On Day -1, subjects will receive 2 test applications of the study drug/placebo, separated by 1 hour. The study drug/placebo will be applied to one eye only (right eye).

Eligibility screening will take place according to the inclusion and exclusion criteria on Day -7 to -1. After screening, the subjects will be randomized to one of the treatment groups. The study drug will be applied to the right eye only. On Day -1, subjects will arrive at the clinical research center for the baseline assessments and will receive instructions on how to apply the study drug. Subjects will receive 2 test applications, separated by 1 hour, to test for tolerability. After an observation time of 15 minutes following the second application, subjects will leave the clinical research center. On Days 0 to 13, study drug will be applied at home. Subjects will return to the clinical research center for ambulant visits on Days 7 and 14. The follow-up medical examination will be performed on Day 21.

SUBJECT POPULATION

The study will be performed in healthy male and female subjects, 18 to 55 years of age, inclusive, with no ocular abnormalities.

DOSAGE AND ADMINISTRATION

Subjects will receive PHMB (0.04%, 0.06% or 0.08%) or placebo 12 times daily (1 drop every hour during day-time) for 7 days (Days 0 to 6) and, if well tolerated, followed by 6 times daily (1 drop every 2 hours during day-time) for an additional 7 days (Days 7 to 13). On Day -1, subjects will receive 2 test applications of the study drug, separated by 1 hour. The study drug/placebo will be applied to the right eye of all subjects.

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PHARMACOKINETIC EVALUATIONS

Blood samples for assessment of PHMB in plasma will be collected on Day 7 and Day 14 upon arrival at the clinical research center.

SAFETY EVALUATIONS

Safety and tolerability assessments will consist of adverse events (AEs), vital signs and clinical laboratory analysis results. Ophthalmologic safety assessments will consist of best corrected visual acuity (BCVA), slit-lamp examination, ocular surface fluorescein and lissamine green staining, ophthalmoscopy, intraocular pressure (IOP), assessment of ocular discomfort and global subjective tolerance (ocular surface disease index [OSDI]).

STATISTICAL METHODS

Sample Size Determination

A total number of 80 evaluable subjects (24 per PHMB treatment group and 8 in placebo group) is considered sufficient for the purpose of the present study for the following reasons:

Based on the binomial probability distribution function, if no serious adverse events (SAEs) are observed in a group of 24 subjects, it can be concluded (with 95% confidence) that the true SAE rate is unlikely to be higher than 11.8%.

For other Dose-Limiting events (DLE), the precision of the study in estimating the DLE rate from a sample of 24 subjects (24 eyes) is shown in the table below:

True DLE rate	Study estimate of the upper 95% confidence bound for DLE *	Sampling error *
15%	29.3%	14.3%
20%	36.0%	16.0%
40%	59.6%	19.6%

* Calculations based on the 'normal' approximation. Probability of not exceeding the sampling error=0.95

Assuming a dropout rate of 10%, the study will aim to recruit 90 subjects.

Pharmacokinetics

Plasma PHMB concentrations will be presented in a descriptive way.

Safety

AEs data will be listed and encoded by lowest level term (Medical Dictionary for Regulatory Activities [current version]). A listing of AEs will be created. This listing, at minimum, will contain a description of AEs as to nature, severity, onset date and end date, duration, therapy (if any), outcome and likelihood of drug causation (“relation"). A frequency table will be compiled for AEs by treatment group, body system, and lowest level term. Frequency tables will be compiled showing the number of subjects per treatment group affected by one or more AEs, including percentages, the total number of AEs per treatment group and the average number of AEs per subject exposed to the respective treatment. Other safety results (vital signs, clinical laboratory analysis results, BCVA, slit-lamp examination, ocular surface fluorescein and lissamine green staining, ophthalmoscopy, IOP, OSDI) will be regarded as descriptive only. P-values will be calculated to flag safety and tolerability variables worthy of further attention.

Safety and tolerability of 3 different concentrations will be compared to placebo.

=> 3 null hypotheses:

- No difference between dose 1 and placebo
- No difference between dose 2 and placebo
- No difference between dose 3 and placebo

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All statistical tests will be performed two-sided and with a 5% significance level. 95% confidence interval for the difference between respective dose and placebo and p-value will be calculated. The focus will be on point estimate and confidence limits.

Which test to be used depends on the type of variable, e.g. logistic regression for binary variables, proportional odds model for an ordinary scaled variable, analysis of variance for continuous variables as appropriate. If the underlying assumptions are not fulfilled, data transformation or non-parametric test will be performed.

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

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SCHEDULE OF ASSESSMENTS

Visits	1 Screening	2		3		4	5 Follow-up
Study Day	**-7 to -1**	**-1[1]**	**0-6**	**7[2]**	**8-13**	**14[2]**	**21**
Study Procedure							
Screening/Administrative							
Informed consent	x						
Demographics	x						
Review medical history	x						
Inclusion/exclusion criteria	x						
Medication history	x						
Urine pregnancy test (women of childbearing potential only)	x						
Study Drug Administration							
Randomization		x[3]					
Dispense study drug[4]		x		x			
Study drug administration at clinical research center[5]		x		x			
Study drug administration at home[6]			x	x	x		
Safety Assessments							
Vital signs[7]	x	x[3]		x		x	x
Hematology, biochemistry[8]	x	x[3]		x		x	x
Urinalysis[8]	x	x[3]		x		x	x
Ocular Safety Assessments							
Best corrected visual acuity	x			x		x	x
Slit-lamp examination[9]	x			x		x	x
Ocular surface fluorescein and lissamine green staining[10]	x			x		x	x
Ophthalmoscopy[11]	x			x		x	x
Intraocular pressure	x			x		x	x
Assessment of ocular discomfort	x			x		x	x
Global subjective tolerance using the Ocular Surface Disease Index grading scale	x			x		x	x
Pharmacokinetics							
Blood sample for PHMB plasma level[12]				x		x	
Ongoing Subject Review							
Concomitant therapy	x	x	x	x	x	x	x
Adverse events	x	x	x	x	x	x	x

1. In case screening will be performed on Day -1, baseline assessments which are planned on Day-1 do not have to be repeated.
2. On Days 7 and 14, subjects will enter the clinical research center and will leave after completion of all assessments.

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3. Before first study drug application.
4. Study drug will be provided for 7 days plus one day spare
5. On Day -1, subjects will receive 2 test applications, separated by 1 hour, to test for tolerability. After an observation time of 15 minutes following the second application, subjects will leave the clinical research center. On Day 7, subjects will visit the clinical research center for assessments. At that time, subjects will apply the study drug themselves at the clinical research center.
6. On Days 0 to 6, subjects will apply study drug 12 times daily (1 drop every hour, daytime). The first dose of the day will be applied on waking up; thereafter, study drug will be applied every hour until 12 doses have been applied. On Days 7 to 13, subjects will apply study drug 6 times daily (1 drop every 2 hours, daytime). The first dose of the day will be applied on waking up; thereafter, study drug will be applied every 2 hours until 6 doses have been applied.
7. Blood pressure and heart rate. Weight and height will be measured at screening only.
8. Subjects should have been fasting for at least 4 hours.
9. Including assessments of abnormalities of conjunctiva, cornea, lens, and anterior chamber.
10. Including assessments of abnormalities of cornea and conjunctiva.
11. Including assessments of abnormalities of vitreous, retina, macula and choroid.
12. Blood sample will be taken on arrival of the subject at the clinical research center.

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ABBREVIATIONS

AE	Adverse event
BCVA	Best corrected visual acuity
CRF	Case report form (paper or electronic as appropriate for this study)
GCP	Good Clinical Practice
DLE	Dose-limiting event
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IOP	Intraocular pressure
IRB	Institutional Review Board
ODAK	Orphan Drug for *Acanthamoeba Keratitis*
OSDI	Ocular Surface Disease Index
PHMB	Polyhexamethylene biguanide
PK	Pharmacokinetic
QPPV	Qualified person responsible for pharmacovigilance
SAE	Serious adverse event
SOC	System organ class

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1. INTRODUCTION

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

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

1.1. Background

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

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

1.1.1. Toxicology

Pure or powder PHMB induced irreversible ocular damage and was considered as corrosive to the rabbit eye, whereas a 20% solutions induced conjunctivitis, corneal opacity, iritis and was considered as moderately irritating to the eye.[7,8] On the basis of all toxicological data currently available, PHMB is considered safe when used up to 0.3%.[7]

Concentrations of 0.05% and a 0.1% PHMB solution have been tested in an irritation test on enucleated pig eyes without causing desquamation of epithelial cells or damage to the microvilli.[9] In addition the administration of a 0.2% PHMB 8 times a day for 4 weeks into the conjunctival sac of the rabbit eye caused mild transient conjunctival redness and discharge. However, the above toxicity signs were not observed if administered 2 times daily.[9] Accordingly,

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PHMB (0.1% and 0.2%) was categorized as non-irritant (Draize test) after a twice daily application over a period of 28 days in the rabbit.[9]

No quantitative or qualitative susceptibility was evident in the available prenatal developmental toxicity studies. Moreover, a two-generation reproductive study did not show quantitative or qualitative evidence of increased susceptibility to abnormalities in the offspring when adults were exposed to PHMB. No mutagenic or genotoxic responses were observed. In EPA 2012, PHMB is classified in the category "Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential" for inciting vascular tumors in laboratory animals by the oral and dermal routes.[8]

To date, the use of PHMB has been shown to be well tolerated and safe in ophthalmology, dentistry[10] and gynecology.[11] Moreover, 0.02% PHMB topical toxicity has been reported rarely and it is limited to mild punctuate keratitis.[12]

1.1.2. Clinical Studies

One prospective, randomized comparative study was conducted to compare the therapeutic efficacy of two monotherapy agents, PHMB and chlorhexidine, in the treatment of *Acanthamoeba keratitis*. According to the Cochrane Database Review, this is to date the only randomized controlled study conducted in patients with *Acanthamoeba keratitis*.[13] Fifty-six eyes of 55 patients with *Acanthamoeba keratitis* were randomized to receive 0.02% PHMB or 0.02% chlorhexidine. Treatments were started immediately after a clinical diagnosis of *Acanthamoeba keratitis* was established. PHMB 0.02% or chlorhexidine 0.02% was commenced at a frequency of hourly day and night for the first two days, then frequency was reduced to hourly by day only for the next five days. The frequency of instillation was further reduced to four times daily according to the clinical response to treatment over succeeding weeks. The results showed that 78% PHMB patients were treatment successes compared with 85.7% chlorhexidine patients (p=0.71). No serious toxic side effects occurred in any of the eyes in the study.[14] Results yielded no difference with respect to outcomes reported between PHMB and chlorhexidine. However, the sample size was small to detect clinically meaningful differences, as indicated by the wide confidence intervals of effect estimates.

For comprehensive nonclinical and clinical information, refer to the Investigator's Brochure for PHMB.[15]

1.2. Overall Rationale for the Study

The ODAK project consortium is investigating PHMB as a safe and effective drug for the treatment of *Acanthamoeba keratitis*. If left untreated, the condition results in blindness. There are currently no approved drugs to treat *Acanthamoeba keratitis*.

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2. OBJECTIVES AND HYPOTHESIS

2.1. Objectives

The primary objective of the study is to establish the ocular safety and tolerability, and systemic safety of 3 different concentrations of preservative-free PHMB in healthy subjects. Safety and tolerability will be compared to those of a placebo.

The PHMB bioavailability in plasma will also be assessed.

2.2. Hypothesis

No formal hypothesis testing is planned.

3. STUDY DESIGN AND RATIONALE

3.1. Overview of Study Design

3.1.1. Type of Study

This is a randomized, double-masked, placebo-controlled, multiple center, parallel-group Phase 1 study to evaluate the safety and tolerability of 3 doses of preservative-free PHMB ophthalmic solution compared to placebo in healthy male and female subjects. The study consists of an eligibility screening visit, 1 treatment period including short ambulant visits, and a follow-up visit.

In total 90 subjects will be assigned to one of the following 4 treatment groups in a ratio of 3:3:3:1.

Group 1: 0.04% PHMB, n=27

Group 2: 0.06% PHMB, n=27

Group 3: 0.08% PHMB, n=27

Group 4: placebo, n=9

In each group, subjects will receive the study drug/placebo 12 times daily (1 drop every hour, daytime only) for 7 days and, if well tolerated, followed by 6 times daily (1 drop every 2 hours, daytime only) for an additional 7 days.

Safety and tolerability evaluations will consist of adverse event (AE) reporting, clinical laboratory (hematology, biochemistry and urinalysis), vital signs and ocular safety evaluations. Blood samples for assessment of PHMB in plasma will be collected on Day 7 and Day 14.

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3.1.2. Screening Visit

Subjects will report to the research center for the eligibility screening according to the inclusion and exclusion criteria defined in Section 4.1 within 1 week prior to the scheduled first drug administration.

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

Eligibility screening will consist of assessments as presented in the schedule of assessments (page 8).

3.1.3. Treatment Period

On Day -1, subjects will arrive at the clinical research center for the baseline assessments and will receive instructions on how to apply the eye drops. Subjects will be randomized to 1 of the 4 treatment groups. The study drug/placebo will be applied to the right eye of all subjects. Subjects will receive 2 test applications, separated by 1 hour, to test for tolerability. After an observation time of 15 minutes following the second application, subjects will leave the clinical research center. On Days 0 to 13, study drops (study drug or placebo) will be self-administered at home. Subjects will return to the clinical research center for ambulant visits on Days 7 and 14.

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

3.1.4. Follow-up

The follow up medical examination will be performed on Day 21. The specific procedures that will be performed are presented in the schedule of assessments.

3.2. Study Design Rationale

A placebo control will be used to establish the frequency and magnitude of changes in endpoints that may occur in the absence of active treatment. Randomization will be used to minimize bias in the assignment of subjects to treatment groups, to increase the likelihood that known and unknown subject attributes (e.g., demographic and baseline characteristics) are evenly balanced across treatment groups, and to enhance the validity of comparisons across treatment groups. Masked treatment will be used to reduce potential bias during data collection and evaluation of endpoints.

4. SUBJECT POPULATION

The study will be performed in healthy subjects.

Screening will be performed between 7 and 1 day before administration of the study drug on Day 0.

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4.1. Inclusion Criteria

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

1. Subject must be able and willing to give informed consent.
2. Subject must be a man or woman of any race and 18 to 55 years of age, inclusive.
3. Subject's Body Mass Index must be 20-30 kg/m^2.
4. Subject must be willing and able to attend required study visits.
5. Subject must have intraocular pressure (IOP) 14-21 mmHg.
6. Subject's ophthalmologic examination must be without clinically significant abnormalities.
7. Subject's medical history is without major pathology.
8. Subject's laboratory test results must be without clinical significant deviations from the normal range.
9. Female subjects of childbearing potential must have a negative urine pregnancy test and must use effective contraception during the study.

4.2. Exclusion Criteria

Any potential subject who meets any of the following criteria will be excluded from participating in the study.

1. Subject with presence of bacterial ocular infections.
2. Subject with presence of any concomitant ocular pathology, except for the presence of bilateral visual acuity between 6/10 and 10/10.
3. Subject performs activities likely to result in an irritated conjunctiva during the study (including heavy alcohol intake, swimming in chlorinated water and heavy smoking).
4. Subject wearing contact lenses at screening until follow-up.
5. Subject who used topical or systemic antibiotics, antihistamines, decongestants and non-steroidal anti-inflammatory agents as well as steroids within 7 days before screening.
6. Subject with known or suspected allergy to biguanides or intolerance to any other ingredient of the test treatments.
7. Subject who underwent ocular surgery.
8. Subjects participating in another clinical study or who had participated in a clinical study in the preceding 30 days.
9. Subjects who have only one functional eye.
10. Female subject being pregnant or breastfeeding.
11. Use of recreational drugs.

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

4.3. Previous / Concomitant Medication and Other Restrictions during Study

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

1. Female subjects of childbearing potential have to use effective contraception during the study.
2. During the study, subjects are not allowed to swim in chlorinated water. In addition, heavy alcohol intake (more than 2 units of alcohol per day for men and 1 unit of alcohol per day for women [1 unit of alcohol equals approximately 250 mL of beer, 100 mL of wine or 35 mL of spirits]) and heavy smoking (more than 20 cigarettes per day) is not allowed during the study.
3. Subjects are not allowed to wear contact lenses at any time from screening to end of follow-up.
4. Subjects are not allowed to use topical or systemic antibiotics, antihistamines, decongestants and non-steroidal anti-inflammatory agents and, as well as steroids within 7 days prior to study entry and during the study.

Concomitant medication in use at the time of initial screening or that is started during the study, will be reported in the case report form (CRF). The name of the drug, the dose and dosage regimen will be recorded.

4.4. Subject Completion/Withdrawal

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

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

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If the subject discontinues, all the final visit assessments must be performed. Moreover, the investigator should ensure that all used and unused study drug for the study period has been collected from the subject. The subject will exit the study and be treated at the Investigator's discretion. Discontinued subjects will not be replaced.

Subjects discontinued for (S)AE(s) will be followed-up after subject's discontinuation until the event is resolved or considered medically stable by the investigator. If necessary, the investigator may schedule further visits at his/her discretion. Data collected in the period up to Day 21 (time of follow-up visit) will be recorded in the CRF. Data collected after Day 21 and related to the (S)AE that led to discontinuation will be recorded in the subject's medical record, and in the sponsor's Safety Database.

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

A subject will be considered to have completed the study if all required assessments up to and including Day 21 of the study have been completed.

5. TREATMENTS

5.1. Treatment Administered

Each subject will be randomly assigned to one of the following 4 treatment groups in a ratio of 3:3:3:1.

Group 1: 0.04% PHMB, n=27

Group 2: 0.06% PHMB, n=27

Group 3: 0.08% PHMB, n=27

Group 4: placebo, n=9

Subjects will receive PHMB (0.04%, 0.06% or 0.08%) or placebo 12 times daily (1 drop every hour during day-time) for 7 days (Days 0 to 6) and, if well tolerated, followed by 6 times daily (1 drop every 2 hours during day-time) for an additional 7 days (Days 7 to 13). On Day -1, subjects will receive 2 test applications of the study drug, separated by 1 hour.

The study drug/placebo will be applied to the right eye of all subjects. On Day -1, study medication will be administered at the research center after completion of all baseline assessments and randomization. The first application will be done by the investigator; the second application will be done by the subject under supervision of the investigator. On Days 0 to 13, study drug will be applied at home and the first dose of the day has to be applied on waking up. Thereafter, study drug will be applied every hour until 12 doses have been applied (Days 0 to 6) or every 2 hours until 6 doses have been applied (Days 7 to 13).

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If the subject misses a dose of study drug, the subject should wait and take the next dose according to the treatment schedule.

Before administration, the single use vials should be shaken vigorously. One drop of study drug should be applied to one eye as instructed by the investigator or research center staff.

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

5.2. Identity of Investigational Product

1. : Test preparation
Name : PHMB
Active compound : polyhexamethylene biguanide
Activity : Cationic surface-active antimicrobial agents
Indication : *Acanthamoeba keratitis*
Strength : 0.04%, 0.06% and 0.08%
Dosage form : eye-drops solution
Manufacturer : SIFI SpA

2. : Reference preparation
Name : placebo
Active compound : NA
Activity : NA
Indication : NA
Strength : NA
Dosage form : eye-drops solution
Manufacturer : SIFI SpA

The content of the study drug formulations is provided in Table 1.

Table 1 Content of the Study Drug Formulations

Component	**% w/v**	**% w/v**	**% w/v**	**Function**
PHMB	0.040 g	0.060 g	0.080 g	Active
$NaH_2PO_4 \cdot H_2O$	0.827 g			Buffer
$Na_2HPO_4 \cdot 12\ H_2O$	0.240 g			Buffer
NaCl	0.520 g			Isotonizer
Purified water	q.s. to 100 mL			Solvent

q.s.: quantity sufficient

5.3. Method of Assigning Subjects to Treatment Groups

After obtaining oral and written informed consent, subjects will be screened according to the inclusion and exclusion criteria. Subjects who have complied with all selection criteria will

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receive a subject number and a randomization number upon enrollment in the study and just prior to dosing.

Subjects will be stratified by site and randomly assigned to Treatment groups 1 to 4 in a 3:3:3:1 ratio based on a computer-generated randomization schedule using 'Random Permuted Blocks' to ensure treatment groups are balanced at end of each block. The schedules are to be prepared before the study by or under the supervision of the sponsor.

Subjects will be assigned to the following treatment groups:

Group 1: 0.04% PHMB, n=27

Group 2: 0.06% PHMB, n=27

Group 3: 0.08% PHMB, n=27

Group 4: placebo, n=9

5.4. Selection of Doses in the Study

There is consensus that 0.02% PHMB eye drops is effective for treatment of *Acanthamoeba keratitis*.[5] However, the efficacy proof of concept data obtained in the rat showed that the use of 0.04%, 0.06% and 0.08% PHMB concentrations were more effective. Both *in vitro* and *in vivo* studies suggest that these concentrations of PHMB are likely to produce little or no more toxicity on the ocular surface than lower concentrations. Since patients with more severe disease, invading the deep stroma, may require a higher concentration of PHMB[6], 3 concentrations of PHMB higher than 0.02% (0.04%, 0.06% and 0.08%) were selected for this study. The highest tolerated dose will be used for the pivotal study in patients.

5.5. Meals during the Study

There are no special requirements with respect to meals during the study, or timing of doses in relation to meals and snacks.

5.6. Masking

This study will be double-masked. The investigator and study staff, the subjects, the monitors, the sponsor will remain masked to the treatment assignment until data collection has been completed, the database is locked and the protocol deviations are determined.

To maintain the double-masked status of the study, the eye drop solution containing test and reference drugs will be indistinguishable in appearance.

The randomization code is kept strictly confidential. Until the moment of unmasking, this code is accessible only to the Qualified Person for Pharmacovigilance of the sponsor.

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Individual code break envelopes will be provided for all subjects and shall be retained by the clinical research centers. Each sealed envelope containing the randomization code will be kept in an area which is locked with restricted access to enable the Investigator to break the code, for safety purposes, when knowledge of the test substance is needed to manage the subject's condition. If opened, the name of the person who opened it, the date and time of opening and the reason for opening must be written on the envelope. The date, time and reason for breaking the code during the study will also be recorded in the subject's CRF.

Before the randomization codes for the double-masked treatment are broken, all sealed code envelopes will be returned to the sponsor.

5.7. Drug Accountability

All drug supplies must be kept in a locked room that can be accessed only by the pharmacist, the investigator, or another duly designated person. This study drug must not be used outside the context of this protocol. Under no circumstances should the investigator or site personnel supply study drug to other investigators or clinics, or allow the supplies to be used other than as directed by this protocol without prior authorization from the sponsor.

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

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

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

6. STUDY EVALUATIONS

6.1. Study Procedures

6.1.1. Overview

The schedule of assessments, presented on page 8, summarizes the frequency and timing of assessments applicable to this study.

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6.1.2. Pharmacokinetic Measurements

The day after each administration schedule (Days 7 and 14), a blood sample will be taken for the analysis of PHMB in plasma. The blood sample will be taken as soon as the subjects have arrived in the clinical research center, preferably in the morning. A blood sample of 2 mL each will be taken by direct venipuncture into heparin tubes. The exact times of blood sampling will be recorded in the CRF, as will the time and date of the last eye drop administered.

6.1.3. Safety and Tolerability Measurements

Safety and tolerability assessments will consist of AEs, vital signs and clinical laboratory analysis results. Ophthalmologic safety assessments will consist of best corrected visual acuity (BCVA), slit-lamp examination, ocular surface fluorescein and lissamine green staining, ophthalmoscopy, IOP, assessment of ocular discomfort and global subjective tolerance (ocular surface disease index [OSDI]). Assessments will be performed in accordance with the schedule of assessments. Between clinical research centers, assessments will be standardized as much as possible.

Adverse Events

Recording of AEs will commence with signing of the ICF. AEs will be captured in the CRF. AEs will be followed up by the investigator as specified in Section 8, Adverse Event Reporting.

Vital Signs

Blood pressure and heart rate measurements will be assessed with the subject in supine position after 5 minutes of rest. Weight and height will be measured at screening only.

Clinical Laboratory Tests

Blood samples for serum chemistry and hematology and a mid stream urine sample for urinalysis will be collected at the time points indicated in the schedule of assessments fasting at least 4 hours. The clinical laboratory will clearly mark all laboratory test values that are outside the normal range and the investigator will indicate which of these deviations are clinically significant or not. The investigator must record all findings during the study that are outside the normal range in the AE section of the CRF.

The following tests will be performed:

- Hematology: full blood count (total red blood cell count / white blood cell count with differential), hematocrit, hemoglobin, platelets.
- Blood biochemistry: serum creatinine, aspartate aminotransferase, alanine aminotransferase, glucose, serum electrolytes (sodium, potassium), total protein, total bilirubin, alkaline phosphatase, gamma-glutamyltransferase, cholesterol and triglycerides.
- Urinalysis (by dipstick): specific gravity, pH, protein, glucose, ketones, urobilinogen, blood.

The following laboratory tests will be performed only at screening:

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- Pregnancy testing in urine (for women of childbearing potential only)

6.1.3.1. Ocular Safety

All ocular safety tests will be performed in both eyes of the subject.

BCVA

BCVA will be determined and recorded at all visits. The same optotypes will be used throughout the study for all subjects.

Assessment of IOP

IOP will be measured using a calibrated tonometer. IOP will be measured and recorded twice consecutively. If the 2 readings are >2 mmHg from each other, a third (consecutive) reading will be taken.

Assessment of ocular discomfort (foreign body sensation, burning/stinging, itching, ocular pain, sticky feeling, blurred vision, and photophobia)

Each symptoms of ocular discomfort (unrelated to instillation of the study drug), including foreign body sensation, burning/stinging, itching, ocular pain, sticky feeling, blurred vision, and photophobia, will be assessed by the subject using a VAS ranging from 0% to 100%. (see Appendix 2). The ocular discomfort score will be calculated as the mean of the VAS scores for each subject. A decrease in the value from baseline will indicate improvement.

Global subjective tolerance assessed by the OSDI grading scale

The OSDI© questionnaire will be used to evaluate the impact of the study drug on the subject's visual functioning. An example of the questionnaire is provided in Appendix 1. All 12 questions will be scored from 0 (none) to 4 (all the time); in addition, 7 questions may be answered as "N/A." The OSDI formula is applied:

$$\text{OSDI}^{©} = \frac{\text{(sum of scores) x 25}}{\text{(\# questions answered excluding questions answered "N/A")}}$$

Thus, overall scores will range from 0, representing no disability, to 100, or complete disability. A negative change from baseline will indicate an improvement in vision-related functioning.

Ophthalmoscopy (including assessments of abnormalities of vitreous, retina, macula and choroid)

Ophthalmoscopy will be performed after pupil dilatation to examine the vitreous, retina, macula and choroid. At screening and baseline, any abnormalities and pathological findings will be recorded in the CRF and graded mild, moderate or severe. At the visits on Days 7, 14 and 21, any new findings or deterioration in baseline findings will be reported as an AE. The cup/disc ratio will be recorded horizontally and vertically for each examination.

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Slit-lamp examination (including assessments of abnormalities of lens, conjunctiva and anterior chamber)

External examination and biomicroscopy will be performed using a slit lamp. Magnification will be consistent with standard clinical practice. The subject will be seated while being examined. Lens will be assessed as normal or abnormal, conjunctiva and anterior chamber will be graded as follows:

Conjunctiva – Erythema

0 = None (normal).
1 = Mild (a flush reddish color predominantly confined to the palpebral or bulbar conjunctiva).
2 = Moderate (more prominent red color of the palpebral or bulbar conjunctiva).
3 = Severe (definite redness of palpebral or bulbar conjunctiva).

Conjunctiva - Edema

0 = None (normal).
1 = Mild (slight localized swelling).
2 = Moderate (moderate/medium localized swelling or mild diffuse swelling).
3 = Severe (severe diffuse swelling).
4 = Very severe (very prominent/protruding diffuse swelling).

Anterior Chamber Inflammation (Slit beam= 0.3 mm wide, 1.0 mm long)

0 = None (no Tyndall effect).
1 = Mild (Tyndall effect barely discernible).
2 = Moderate (Tyndall beam in the anterior chamber is moderately intense).
3 = Severe (Tyndall beam in the anterior chamber is severely intense).

Assessment of corneal abnormalities

Assessment of corneal abnormalities before vital staining of the ocular surface

Cornea: the presence or absence of the following will be recorded:

Corneal epithelial defects
Corneal ulceration
Corneal epithelial opacity/infiltrate
Corneal stromal opacity/infiltrate
Other

Conjunctiva: the presence or absence of the following will be recorded:

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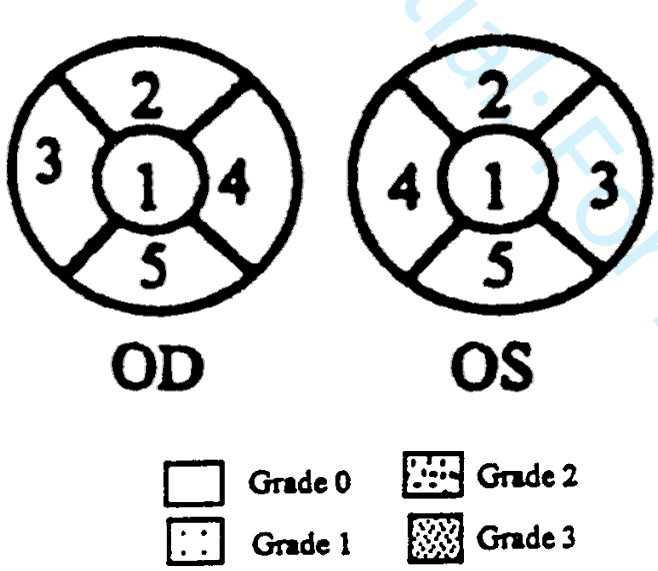
Discharge
Papillae
Follicles

Vital staining of the ocular surface

A fluorescein strip (Fluoret™, Chauvin Pharmaceuticals, Bausch and Lomb) will be wet with a single drop of non-preserved saline solution and the stained drop will be transferred from the strip to the inferior palpebral conjunctiva. The subject will be asked to close their eyelids and move their eyes to distribute the fluorescein in the tear film. Surface must be assessed in 1-3 minutes.

Cornea: the cornea will be examined immediately after the fluorescein instillation using the cobalt blue light on the slit lamp. The density of punctuate staining will be graded from 0 to 3 in each of the 5 areas of the cornea, using the scale and diagram shown below. The results will be summed to produce a result out of 15.



OD: oculus dextrus, right eye; OS: oculus sinister, left eye

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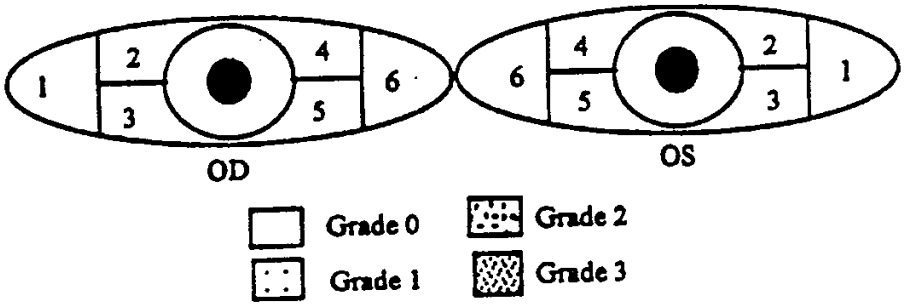
Conjunctiva: After 10 minutes, a lissamine green strip will be wet with a single drop of non-preserved saline solution and the stained drop then transferred from the strip into the lower palpebral conjunctival tear film. The subject will be asked to close their eyelids and move their eyes to distribute the dye. After 15 seconds the conjunctiva will be examined with the green light on the slit lamp. The density of punctate staining will be graded from 0 to 3 in each area of the conjunctiva for each eye. The results will be recorded on the diagram as presented below and summed to produce a score out of a total of 18 for each eye.



OD: oculus dextrus, right eye; OS: oculus sinister, left eye

6.2. Appropriateness of Measurements

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

6.2.1. Timing of Assessments

The actual times of all assessments will be recorded in the CRF.

Pre-dose assessments will be performed between waking up and first dosing.

In the event assessments are planned for the same scheme time, the order of the assessments should be arranged in such a way that vital signs will be done first, followed by pharmacokinetic (PK) blood sampling, clinical laboratory, and other safety assessments.

6.3. Primary Variables

The primary variables to be evaluated will be those relating to Dose-Limiting Events (DLE), including severe life-threatening or blinding events (serious adverse events [SAEs]).

6.4. Pharmacokinetic Variables

The PK parameter to be determined is the PHMB plasma concentration on Days 7 and 14.

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6.5. Analytical Procedures

Plasma samples will be stored at -20°C in controlled conditions at the study site during the development and validation of the method of analysis of PHMB (high-performance liquid chromatography–mass spectrometry).

6.6. Sample Collection and Handling

The actual dates and times of sample collection must be recorded in the CRF.

Refer to the schedule of assessments for the timing and frequency of all sample collections. Details on blood sampling procedures can be found in the lab manual which will be provided to the study sites.

7. STATISTICAL METHODS

7.1. Study Populations

Population for the pharmacokinetic analyses

All subjects and all available PK-data will be analyzed in the pharmacokinetic analyses.

Population for the safety analysis

Safety data for all subjects who have received at least 1 dose of study medication will be analyzed in the safety analyses.

7.2. Statistical and Analytical Plan for Pharmacokinetic and Safety and Tolerability Evaluation

A Statistical Analysis Plan will be generated by the study statistician, which will be finalized prior to the breaking of the mask of the study.

All statistical analyses will be performed using the computer program SAS® latest version available.

7.3. Demographic Variables and Baseline Characteristics

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

7.4. Pharmacokinetic Evaluation

Plasma PHMB concentrations will be presented in a descriptive way.

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7.5. Evaluation of Safety and Tolerability Parameters

7.5.1. Adverse events

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

7.5.2. Other Safety Parameters

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

Safety and tolerability of 3 different concentrations will be compared to placebo.

=> 3 null hypotheses:

- No difference between dose 1 and placebo
- No difference between dose 2 and placebo
- No difference between dose 3 and placebo

All statistical tests will be performed two-sided and with a 5% significance level. 95% confidence interval for the difference between respective dose and placebo and p-value will be calculated. The focus will be on point estimate and confidence limits.

Which test to be used depends on the type of variable, e.g. logistic regression for binary variables, proportional odds model for an ordinary scaled variable, analysis of variance for continuous variables as appropriate. If the underlying assumptions are not fulfilled, data transformation or non-parametric test will be performed.

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

7.6. Sample Size Determination

A total number of 80 evaluable subjects (24 per PHMB treatment group and 8 in placebo group) is considered sufficient for the purpose of the present study for the following reasons:

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Based on the binomial probability distribution function, if no SAEs are observed in a group of 24 subjects, it can be concluded (with 95% confidence) that the true SAE rate is unlikely to be higher than 11.8%.

For other DLE, the precision of the study in estimating the DLE rate from a sample of 24 subjects (24 eyes) is shown in the table below:

True DLE rate	Study estimate of the upper 95% confidence bound for DLE *	Sampling error *
15%	29.3%	14.3%
20%	36.0%	16.0%
40%	59.6%	19.6%

* Calculations based on the 'normal' approximation. Probability of not exceeding the sampling error=0.95

Assuming a dropout rate of 10%, the study will aim to recruit 90 subjects.

7.7. Interim Analysis

There will be no interim analysis.

7.8. Data Monitoring Committee

Not applicable.

8. ADVERSE EVENT REPORTING

8.1. Definitions

8.1.1. Adverse Event

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

AEs include the following:

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

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

- Overdose
- Interactions
- Abuse
- Misuse

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8.1.2. Abnormal test findings

An abnormal test finding, e.g., abnormal laboratory analysis results or vital signs should be recorded as an AE in any of the following situations:

- The test is associated with accompanying symptoms. Note, that the symptom, not the test result, should be recorded as an AE.
- The test result leads to a medical/surgical intervention including withdrawal of study drug or discontinuation from the study. Repeat/confirmatory testing is not considered a medical intervention.
- The investigator considers the test result to be clinically significant.

8.1.3. Pre-existing conditions

A pre-existing condition (i.e., a disorder present before the AE reporting period started and noted on the pre-treatment medical history form) should not be reported as an AE unless the condition worsens or episodes increase in frequency during the AE reporting period.

8.1.4. Procedures

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

8.1.5. Serious Adverse Events

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

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

Other medically important AEs that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in

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hospitalization or development of dependency or abuse. Serious also includes any other event that the investigator or company judges to be serious. Any suspected transmission of an infectious agent via the study drug shall also be considered serious.

8.1.6. Hospitalization

Hospitalization includes transfers within a hospital (e.g., from the psychiatric unit to the intensive care unit) and also includes admissions less than 24 hours. The following situations are not considered hospitalizations (although other SAE criteria may still apply):

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

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

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

8.2. Adverse Event Reporting Period

The period for recording AEs, including SAEs, on the CRF begins upon the subject has signed the informed consent (Visit 1) and ends at the last study visit (Visit 5). In addition, SAEs should be reported until 28 days past the last dose of study drug. SAEs that are related to the study drug and continue beyond the normal collection period (i.e., are ongoing) will be followed until the SAE is resolved or considered medically stable by the investigator. Furthermore, any SAE should be reported irrespective of the time of occurrence if a causal relationship between the event and the study drug is suspected. Data collected after Day 21 (follow-up visit) will be recorded in the subject's medical record, and in the sponsor's Safety DatabaseEliciting and Recording Adverse Event Information

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

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

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8.2.1. Severity Assessment

The intensity of AEs will be graded using the most current version of the Common Terminology Criteria for Adverse Events five-point scale:

- **Mild (Grade I)**: Asymptomatic or mild symptoms: clinical or diagnostic observations only; intervention not indicated.
- **Moderate (Grade II)**: Minimal, local or non-invasive intervention indicated; limited age-appropriate instrumental activities of daily living.
- **Severe (Grade III)**: Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living; incapacitating with inability to work or perform normal daily activity.
- **Life-threatening (Grade IV)**: consequences: urgent intervention indicated.
- **Death (Grade V)** related to AE.

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

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

8.2.2. Causality Assessment

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

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

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

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

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

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

Related: As for "probable" - but AE reappears on repeated exposure (re-challenge).

8.3. Serious Adverse Event Reporting

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

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

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

All new information obtained, relevant to an SAE report, should be forwarded to the Monitor and the Sponsor's QPPV within the same timeframe as the initial information.

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

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

Daria Rasà
Qualified Person Responsible for Pharmacovigilance
Tel +39 095 79 22 204
Mob +39 346 8399737
Fax +39 095 78 93 451
e-mail: daria.rasa@sifigroup.com

or

Michele Puglia
Deputy QPPV
Tel: +39 095 79 22 356

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Mob: +39 335 60 77 919
E-mail: michele.puglia@sifigroup.com

Where required by local regulations, the IRB/EC and/or regulatory authorities will be informed of SAEs in accordance with local regulation timeframes and reporting requirements.

8.4. Exposure during Pregnancy

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

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

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

8.5. Follow-up of Adverse Events

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

8.6. Recording

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

This will include the following information:

- Description of the AE
- Date and time of onset – End date and time
- Time of last study drug dose
- Severity of symptoms
- Action taken with study drug

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- Action taken (medication)
- AE outcome
- Assessment of relation to study medication
- Comments by investigator

In addition, clinically significant changes in abnormal objective test findings (e.g., laboratory) should also be recorded as AEs. Test findings can result in AEs if they are:

- Associated with accompanying symptoms, and/or
- Require additional diagnostic testing or medical/surgical intervention, and/or
- Lead to a change in study dosing or discontinuation from the study, significant additional concomitant drug treatment or other therapy, and/or
- Lead to any of the outcomes included in the definition of a SAE, and/or
- Considered to be an AE by the Medical Investigator or Sponsor.

Reporting as an AE should not be triggered by:

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

9. QUALITY CONTROL AND QUALITY ASSURANCE

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

The study center may also be subject to review by an independent ethics committee (IEC), to quality assurance audits performed by the sponsor, and/or to inspection by appropriate regulatory authorities.

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

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10. ETHICAL ASPECTS

10.1. Independent Ethics Committee/Institutional Review Board

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

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

10.2. Ethical Conduct of the Study

The study will be performed in accordance with International Conference on Harmonization GCP guidelines, the Declaration of Helsinki (adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, June 1964, and subsequent amendments[16]), and applicable local regulatory requirements and laws.

10.3. Subject Information and Consent

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

10.4. Privacy of Personal Data

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

11. STUDY ADMINISTRATIVE STRUCTURE

11.1. Documentation

11.1.1. Archiving

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

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11.1.2. Case report form

A CRF is required and should be completed for each included subject. In this study an electronic CRF will be used, created within the Viedoc system. Viedoc is a fully validated and Food and Drug Administration (FDA) compliant Electronic Data Capture (EDC) system, with several additional features, such as Interactive Web Response System (IWRS) and the possibility for subjects to complete questionnaires directly into the system.

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

11.1.3. Source data

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

12. CONFIDENTIALITY AND PUBLICATION POLICY

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

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

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

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

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

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

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

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

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

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16 WMA Declaration of Helsinki (18th WMA General Assembly 1964), revised at 64th World Medical Association General Assembly, Fortaleza, October 2013.

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Appendix 1: Ocular Surface Disease Index© Questionnaire

The subject will be asked to answer the following 12 questions, and check the box that best represents his/her answer. Then, fill in boxes A, B, C, D and E according to the instructions beside each box.

Have you experienced any of the following during the last week?	All of the time	Most of the time	Half of the time	Some of the time	None of the time
1. Eyes that are sensitive to light?	4	3	2	1	0
2. Eyes that feel gritty?	4	3	2	1	0
3. Painful or sore eyes?	4	3	2	1	0
4. Blurred vision?	4	3	2	1	0
5. Poor vision?	4	3	2	1	0

Subtotal score for answers 1 to 5: ☐ **(A)**

Have problems with your eyes limited you in performing any of the following during the last week?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
6. Reading?	4	3	2	1	0	N/A
7. Driving at night?	4	3	2	1	0	N/A
8. Working with a computer or bank machine (ATM)?	4	3	2	1	0	N/A
9. Watching TV?	4	3	2	1	0	N/A

Subtotal score for answers 6 to 9: ☐ **(B)**

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Have your eyes felt uncomfortable in any of the following situations during the last week?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
10. Windy Conditions?	4	3	2	1	0	N/A
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	N/A
12. Areas that are air conditioned?	4	3	2	1	0	N/A

Subtotal score for answers 10 to 12: ☐ **(C)**

Add subtotals A, B, and C to obtain D

(D = sum of scores for all questions answered) ☐ (D)

Total number of questions answered

(do not include questions answered N/A) ☐ (E)

OSDI© is assessed on a scale of 0 to 100 using the OSDI formula:

OSDI© score = D (sum of scores) X 25
E (# of questions answered)

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Appendix 2: Visual Analog Scale (Vas) – Symptoms of Ocular Discomfort

The subject will be asked to assess each symptom regarding ocular discomfort unrelated to instillation among a list of 7 symptoms, i.e., foreign body sensation, burning/stinging, itching, ocular pain, sticky feeling, blurred vision, and photophobia. The subject will be asked to rate each ocular symptom by placing a vertical mark on the horizontal line to indicate the level of discomfort. 0% corresponds to "no discomfort" and 100% corresponds to "maximal discomfort."

Only filled out by the subject!	**Date:** |__|__|.|__|__|__|.|__|__|__|__| day month (text) year **Time:** |__|__|:|__|__| (24-h) hh min Right eye ☐ Left eye ☐
Please mark each ocular symptom by placing a vertical mark on the horizontal line between 0 and 100 mm to indicate the intensity of discomfort.	

VISUAL ANALOGUE SCALE (VAS)

This Visual Analogue Scale (VAS) will be used to determine the ocular tolerability in the study eye

Foreign body sensation	no symptoms … worst possible discomfort 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 0mm … 100mm
Burning/stinging	no symptoms … worst possible discomfort 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 0mm … 100mm
Itching	no symptoms … worst possible discomfort 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 0mm … 100mm
Ocular *Pain*	no symptoms … worst possible discomfort 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 0mm … 100mm
Sticky feeling	no symptoms … worst possible discomfort 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 0mm … 100mm
Blurred Vision	no symptoms … worst possible discomfort 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 0mm … 100mm
Photophobia	no symptoms … worst possible discomfort 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 0mm … 100mm

The response will be measured in % between 0 – 100%.

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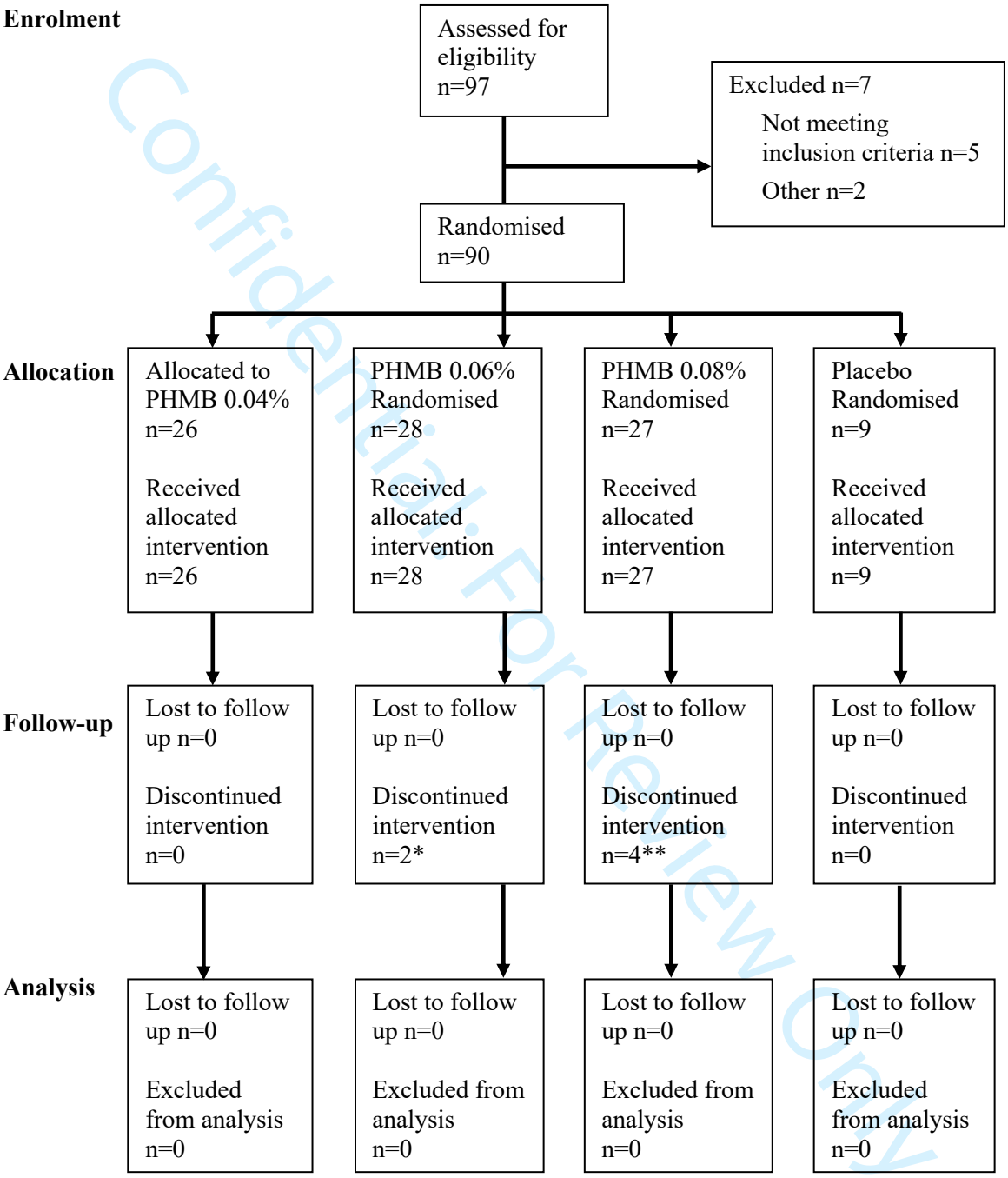
Supplementary Figure 1

Flow diagram of progress of subjects through the Phase 1 study of the "Safety and tolerability of topical polyhexamethylene biguanide (PHMB): a randomised clinical trial in healthy adult volunteers"



* Two subjects developed dose limiting adverse effects see Table 2 and Supp Table 1 for details

** Three of 4 subjects developed dose limiting adverse effects see Table 2 and Supp Table 1 for Details. One of 4 was discontinued after 4 days of treatment because of alcohol consumption in excess of protocol limits. Because all the DLAE developed within 1-4 days of onset it was concluded that this subject was at risk of developing a DLAE and was included in the analysis

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