AMERICAN ACADEMY OF OPHTHALMOLOGY®

# The Orphan Drug for *Acanthamoeba* Keratitis (ODAK) Trial

## *PHMB 0.08% (Polihexanide) and Placebo versus PHMB 0.02% and Propamidine 0.1%*

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***Purpose:*** To compare topical PHMB (polihexanide) 0.02% (0.2 mg/ml)+ propamidine 0.1% (1 mg/ml) with PHMB 0.08% (0.8 mg/ml)+ placebo (PHMB 0.08%) for *Acanthamoeba* keratitis (AK) treatment.

***Design:*** Prospective, randomized, double-masked, active-controlled, multicenter phase 3 study (ClinicalTrials.gov identifier, NCT03274895).

***Participants:*** One hundred thirty-five patients treated at 6 European centers.

***Methods:*** Principal inclusion criteria were 12 years of age or older and in vivo confocal microscopy with clinical findings consistent with AK. Also included were participants with concurrent bacterial keratitis who were using topical steroids and antiviral and antifungal drugs before randomization. Principal exclusion criteria were concurrent herpes or fungal keratitis and use of antiamebic therapy (AAT). Patients were randomized 1:1 using a computer-generated block size of 4. This was a superiority trial having a predefined noninferiority margin. The sample size of 130 participants gave approximately 80% power to detect 20-percentage point superiority for PHMB 0.08% for the primary outcome of the medical cure rate (MCR; without surgery or change of AAT) within 12 months, cure defined by clinical criteria 90 days after discontinuing anti-inflammatory agents and AAT. A prespecified multivariable analysis adjusted for baseline imbalances in risk factors affecting outcomes.

***Main Outcome Measures:*** The main outcome measure was MCR within 12 months, with secondary outcomes including best-corrected visual acuity and treatment failure rates. Safety outcomes included adverse event rates.

***Results:*** One hundred thirty-five participants were randomized, providing 127 in the full-analysis subset (61 receiving PHMB 0.02%+ propamidine and 66 receiving PHMB 0.08%) and 134 in the safety analysis subset. The adjusted MCR within 12 months was 86.6% (unadjusted, 88.5%) for PHMB 0.02%+ propamidine and 86.7% (unadjusted, 84.9%) for PHMB 0.08%; the noninferiority requirement for PHMB 0.08% was met (adjusted difference, 0.1 percentage points; lower one-sided 95% confidence limit, −8.3 percentage points). Secondary outcomes were similar for both treatments and were not analyzed statistically: median best-corrected visual acuity of 20/20 and an overall treatment failure rate of 17 of 127 patients (13.4%), of whom 8 of 127 patients (6.3%) required therapeutic keratoplasty. No serious drug-related adverse events occurred.

***Conclusions:*** PHMB 0.08% monotherapy may be as effective (or at worse only 8 percentage points less effective) as dual therapy with PHMB 0.02%+ propamidine (a widely used therapy) with medical cure rates of more than 86%, when used with the trial treatment delivery protocol in populations with AK with similar disease severity.

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*Acanthamoeba* keratitis (AK), first described only 48 years ago, is one of the less common causes of microbial keratitis, but also one of the most severe. *Acanthamoeba* keratitis requires prolonged treatment times and high rates of surgical intervention and has had poor visual outcomes for one-third of patients,[1−3] accounting for approximately 50% of contact lens users who lose sight as a result of microbial keratitis.[4] The incidence has been increasing, not only in countries with high personal income where contact lens wear is associated with most cases,[3] but also in India, where

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agricultural trauma is the main association.[5] Treatment for AK is an unmet need for which no licensed drugs are available.

Existing data on AK treatment outcomes, from a systematic review of AK treatments from studies for which medical cure rates (without surgery) could be established, are summarized in Appendix 1A–E (available at www.aaojournal.org). Medical cure rates (Appendix 1B) ranged from 33.3% to 100% in 18 studies; with 1 exception, cure rates higher than 70% (13 studies) were from case series with fewer than 30 patients and do not reflect recent concerns that AK has become more difficult to treat.[3,6] Probably more realistic cure rates are reflected in the findings of the 2 largest case series totaling more than 400 eyes, summarized in Appendix 1E, reporting medical cure rates within 12 months for predominantly topical polihexanide (PHMB) treatments in 138 of 227 patients (60.79%), with poor outcomes (acuity $\leq$ 20/80, surgery, or both) in 112 of 227 patients (49.3%),[1] and for medical cures at some time point (undefined) for predominantly topical chlorhexidine treatments in 158 of 224 patients (70.5%),[2] with poor outcomes (acuity $\leq$ 20/40, keratoplasty, or both) in 87 of 224 patients (38.8%).

It is with this background that the prospective, randomized, double-masked, active-controlled, multicenter phase 3 trial reported herein was designed, with European Medicines Agency assistance, as a pivotal clinical trial with the potential to deliver topical PHMB 0.08% monotherapy as the first licensed treatment for AK. A phase 2 study was not required. The formulation has been made to Good Manufacturing Practice (GMP) standards with the necessary safety evaluations, including a phase 1 study showing no clinically significant toxicity in healthy human volunteers.[7] PHMB 0.08% was chosen over lower concentrations as being more likely to improve antiamebic activity by increasing corneal stromal drug concentrations compared with the widely used comparator of PHMB 0.02% with propamidine 0.1% (Brolene, Sanofi-Aventis, UK). We hypothesized that PHMB 0.08% monotherapy would be more effective, or at least as effective, as the dual-therapy comparator.

## Methods

The trial was designed, monitored, and conducted in accordance with Good Clinical Practice guidelines and the tenets of the Declaration of Helsinki. Ethics approvals were obtained before the start at all trial centers (Appendix 2, available at www.aaojournal.org), and all participants gave informed consent. The study was carried out from August 17, 2017 through June 18, 2021, at 6 centers: 3 in the United Kingdom (London, Southampton, and Manchester), 2 in Italy (Milan and Venice), and 1 in Poland (Katowice). Principal and coinvestigators were trained and provided with both the protocol (Appendix 3, available at www.aaojournal.org) and study operations manuals (Appendix 4, available at www.aaojournal.org). Training on in vivo confocal microscopy (IVCM) was undertaken by representatives from each center; all centers used the Heidelberg Retina Tomograph 3 with the Rostock Corneal Module (Heidelberg Engineering GmbH), apart from Katowice, which used the Nidek Technologies Confoscan 4. A contract research organization, the PSR Group BV (subsequently incorporated into Ergomed PLC, United Kingdom), coordinated project management, regulatory submissions, clinical monitoring, maintenance of the trial master file, and data management. The trial was terminated after the last follow-up visit of the prespecified number of participants. Minor ethically approved amendments were made to the protocol (Appendix 3) after the start of the trial.

### Trial Design

This was a prospective, randomized, double-masked, active-controlled, multicenter, parallel-group phase 3 trial to evaluate the efficacy, safety, and tolerability of topical PHMB 0.08% (0.8 mg/ml) monotherapy with placebo compared with PHMB 0.02% (0.2 mg/ml) with propamidine 0.1% (1 mg/ml) dual therapy for the treatment of AK. The PHMB formulations were identical, non-preserved in single-dose units, and included the same excipients. Brolene was obtained from Sanofi-Aventis and contains benzalkonium chloride (BAC) 0.005% as preservative, as did the placebo. SIFI S.p.A. produced both PHMB and placebo eye drops to GMP standards. The trial was designed to reflect the mix of participants with AK seeking treatment at specialist centers to make the findings relevant to most participants with AK internationally, while including only those with a confirmed (as opposed to a clinical) AK diagnosis. Participants with concurrent fungal or herpes keratitis were excluded as having too complex a clinical course to reflect the results of AK treatments. The study comprised an eligibility screening visit combined with consent and randomization for eligible participants and a treatment period with visits at 0, 7, 14, 21, and 30 days and thereafter every 30 days until clinical cure, followed by visits at 30 and 90 days before discharge. Because the eligibility screening visit was combined with consent and randomization, the data collected from participants who declined to participate was poor and was not collected routinely; at Moorfields, 13 of the first 62 patients (21%) with probable AK approached to enter the trial declined to participate.

### Definitions Used in the Trial

- Baseline: Tests could be carried out up to 2 days before day 0 when randomization, clinical assessments, completion of baseline quality-of-life questionnaires, and trial treatment were started.
- Cure: Clinical evidence of elimination of *Acanthamoeba*, including an intact corneal epithelium with no clinical signs or symptoms of ocular inflammation after discontinuing antiamebic therapy (AAT) and anti-inflammatory treatment for 30 days (confirmed at the end-of-study visit 90 days after treatment discontinuation) as determined by clinical examination.
- Medical cure rate (MCR) within 12 months: The rate (proportion) cured (as defined above) by each treatment without the need for surgery or a change of AAT, and independent of visual acuity, within 12 months of randomization. This was the primary outcome measure for the trial.
- Treatment failure: Instances of trial participants for whom other topical or oral AATs were used, in addition to trial medications, or who were changed to an alternative AAT, usually because of deteriorating disease or adverse events, were considered treatment failures. Other reasons were failure to be cured by trial medications in periods longer than 12 months, the need for any type of surgery, a requirement for oral immunosuppressive therapy, and the development of trial drug-related adverse events.

- *Acanthamoeba* keratitis disease staging: Stage I AK, corneal epitheliopathy only; stage II AK, the presence of 1 or more corneal epithelial defects, perineural infiltrates, or stromal infiltrate, in addition to stage 1 findings; and stage III AK, a corneal ring infiltrate and 1 or more features of stage 2 disease.

## Participants

**Principal Inclusion Criteria.** Principal inclusion criteria were as follows. (1) Participants were those of any race and sex who were 12 years of age or older and were enrolled by principal or co-investigators. (2) Participants demonstrated clinical findings consistent with AK: principally corneal epithelial pathologic features (epithelial punctate keratopathy, epithelial infiltrates, epithelial defects, and dendritiform epithelial ulcers), corneal stromal pathologic features (perineural infiltrates, anterior stromal infiltrates, disciform corneal swelling, stromal ulceration, and ring abscess), and extracorneal pathologic features (limbitis and diffuse or nodular anterior scleral inflammation). A full list is included in Appendix 4 (specifically, Appendix 8 within that appendix). (3) Participants demonstrated IVCM findings consistent with AK (polymerase chain reaction [PCR] and culture analysis also were carried out on all participants, but were not used for inclusion or exclusion). For participants with an IVCM diagnosis of AK and negative culture or PCR findings, or both, their IVCM files were reviewed by an expert coinvestigator (S.H.). Patients whose findings did not meet the IVCM criteria[8] for an AK diagnosis were excluded from the full analysis set to minimize the inclusion of false-positive AK diagnoses. This expert review was not included in the protocol, but was instituted before the end of the trial.

Note that participants meeting the above criteria and using the following previous treatments for keratitis (mis)diagnoses also were eligible for the study: antibiotics for a presumed or proven ocular bacterial infection at baseline; antiviral and antifungal drugs given for a misdiagnosis and discontinued at baseline; and anti-inflammatory drugs, including those using topical steroids, oral nonsteroidal anti-inflammatory drugs, or both before baseline. Anti-inflammatory drugs were changed to equivalent study preparations (diclofenac tablets or topical dexamethasone 0.1%) unless contraindicated.

**Principal Exclusion Criteria.** Principal exclusion criteria included the following: (1) pregnancy or inability to use contraception (both men and women) from baseline and for specified periods after the last dose of study drugs; (2) a documented history or clinical signs of concomitant keratitis, or both, caused by herpes simplex virus or fungi; (3) treatment before baseline with anti-amebic agents (PHMB, chlorhexidine, propamidine, and hexamidine); (4) participants using systemic immunosuppressive therapy; and (5) participants requiring urgent surgical intervention for AK.

## Randomization, Interventions, Treatment Delivery Protocol, and Masking

Participants were assigned to 1 of the 2 trial AATs. Each treatment was assigned a unique code allocated by a 1:1 computer-generated block randomization schedule with a block size of 4 prepared by the contract research organization using a statistical service contractor. All contract research organization staff, trial staff, and participants were masked to the treatments throughout the trial. After enrolment by the investigators, treatments were provided through the trial center pharmacies and were assigned by the unique treatment randomization code. Unmasking was carried out only when the study database was locked at the end of study. For participants with bilateral disease, study treatment was allocated to the worst affected eye, unless the eyes were equally severely affected when the right eye was treated; the nonstudy eye was treated with the best treatment according to clinical practice at each study center. Each participant received 2 study drugs, either PHMB 0.08% with placebo or PHMB 0.02%+ propamidine 0.1%. The placebo and propamidine containers were slightly different but because none of the participants were unmasked during the study, masking was maintained. A detailed protocol was used throughout the trial for delivery of both AAT and for adjunctive therapy (Fig S1, from the protocol in Appendix 3, available at www.aaojournal.org). Adherence to this protocol was externally monitored throughout the trial.

## Outcomes

The prespecified primary outcome measure was the MCR within 12 months from randomization. This was chosen because it is arguably the most important outcome measure for both patients and clinicians because of the poor outcomes both of therapeutic and optical keratoplasty for AK.[9] After a medical cure, patients can make informed decisions about the advisability of further surgical treatments to improve their vision in what is often a unilateral disease. Prespecified secondary outcome measures were best-corrected visual acuity, corneal scarring rates, treatment failure rates, and patient-reported outcomes using the EuroQol 5 Dimension 5 Level health related quality of life questionnaire (EQ-5D-5L) and the 25 Item Visual Function Questionnaire (VFQ25) tools. Prespecified safety outcome measures were adverse event reports and clinical laboratory assessments (hematology, biochemistry, and urinalysis) performed at baseline and at the end of the study visit (apart from urine pregnancy tests, which were carried out monthly in premenopausal women). Prespecified secondary safety outcome measures were repeat courses of intensive treatment for presumed relapses of infection, adjunctive topical steroid use, cataract, raised intraocular pressure, severe inflammatory disease onset after baseline (ring abscess, hypopyon, and scleritis), corneal vascularization, and chorioretinal disease.

## Statistical Analysis

**Sample Size Determination.** The study protocol (Appendix 3) planned for 130 participants with AK to be randomized 1:1 to 1 of the 2 treatment groups. The prespecified analysis was a superiority analysis also meeting the requirements for a noninferiority analysis as defined in the European Medicines Agency guidance CPMP/EWP/482/99 (Appendix 5, available at www.aaojournal.org). The power calculation was based on the MCR within 12 months outcomes for PHMB 0.02% treatments in a retrospective study of 100 patients with AK from Milan and London (later expanded to 227 patients and published[1]), carried out to inform the power calculation and design of the phase 3 trial. These data estimated the MCR within 12 months for PHMB 0.02%+ propamidine 0.1% at (57/85) 67%, reduced to 63% to account for an anticipated increase in patients with more advanced (stage III) AK likely to have a poorer outcome. This required a sample size of 116 evaluable participants, requiring 130 participants after allowing for a 10% loss to follow-up. This sample size was required to give approximately 80% power to detect a superiority of 20 percentage points for PHMB 0.08% monotherapy, with a 2-sided $\alpha$ value of 0.10 (or equivalently, a 1-sided $\alpha$ value of 0.05) and a predefined noninferiority margin of 20 percentage points. This sample size and the hypothesized superiority of 20 percentage points for PHMB 0.08% was reached not

only because of the requirement to test for superiority, but also to ensure the adequacy of the sample size for the noninferiority analysis if superiority was not achieved.

**Analyses.** Analyses followed the predefined statistical plan included in the protocol except for the time-to-cure analysis (see below) and were carried out by Link Medical Research in Sweden and Epivision in the United Kingdom. The software package used by Link was SAS software version 9.4 (SAS Institute, Inc.), and that used by Epivision UK was Stata software version 17 (StataCorp LP). The primary outcome measure was the MCR within 12 months. Baseline differences in risk factors between the two treatment arms were not distributed evenly by the randomization because of recruitment numbers being relatively small; a multivariable analysis was prespecified in the protocol (but not the statistical plan) because these differences were anticipated. As a result, multivariable analyses were applied to the primary outcome of the MCR within 12 months and, as a subgroup analysis, the MCR within 12 months outcomes for each AK disease stage at baseline. A Poisson model with robust variance was used to evaluate the unadjusted treatment effect and to estimate the difference in MCR within 12 months between the 2 treatments after adjustment for baseline covariates. Covariates selected as candidates for inclusion in the model-building process were those that were known prognostic factors affecting the outcome of AK (age, AK stage, delay in diagnosis, corticosteroid use before baseline, and antiviral use before baseline) or were suspected prognostic factors, including antibiotic use before baseline and study site (6 coded centers). The Kaplan-Meier curves were adjusted for covariates, as predicted by the Cox proportional hazards model, and were a time-to-eventual-cure analysis that included all the trial failures at the point of a cure from *Acanthamoeba* infection as defined by having been discontinued from AAT. The amount of missing data was considered negligible and too few to allow recognition of a missingness pattern. The few missing days or months in the date fields were imputed to be first day or first month of the year data.

## Results

Recruitment started on August 17, 2017, with the last visit completed June 18, 2021, and totaled 135 participants. The Consolidated Standards of Reporting Trials diagram (Fig 2) shows the treatment allocations for the safety analysis (n = 134), full analysis (n = 127), and per-protocol analysis (n = 119). Baseline data for the full analysis subset are shown in Table 1 with covariates used in the adjusted analyses marked with an asterisk. Baseline data for the 135 randomized participants are provided in Table S2 (available at www.aaojournal.org). Diagnostic test results are shown in Table 3. PHMB 0.02% plus propamidine 0.1% is subsequently abbreviated to PHMB 0.02%+ and PHMB 0.08% with placebo to PHMB 0.08%.

### Primary Outcome Analysis (Prespecified Primary Analysis)

Table 4 gives the results for the MCR within 12 months. The crude (unadjusted for the confounding effects of baseline risk factors) outcomes were cure rates of 54 of 61 participants (88.52%) for PHMB 0.02%+ and 56 of 66 (84.85%) for PHMB 0.08% (exact $P = 0.609$). The adjusted final analysis gives rates of 86.55% for PHMB 0.02%+ and 86.68% for PHMB 0.08% (exact $P = 0.980$). The per-protocol crude analysis (Table S5, available at www.aaojournal.org) was very similar at 51 of 57 participants (89.47%) for PHMB 0.02%+ and 54 of 62 participants (87.10%) for PHMB 0.08%. The prespecified noninferiority margin for PHMB 0.08%, compared with the comparator, of 20 percentage points has been met, with a lower boundary of the 1-sided 95% confidence interval of −13.6 percentage points in the unadjusted analysis and −8.3 percentage points in the adjusted analysis (Table S6, available at www.aaojournal.org).

**Secondary Outcome Measures.** Secondary outcome measures are summarized in Table 7. Because no meaningful differences between the two treatment arms were found for any of the secondary outcomes, these were not analyzed statistically and neither *P* values nor confidence limits were added. However, full data for best-corrected visual acuity, treatment failures, and quality-of-life scores are given in Tables S8, S9, and S10 (available at www.aaojournal.org), respectively. Of note were the outcomes for best-corrected visual acuity in Table S8, with median acuities of 20/20 for both treatment arms (interquartile ranges, 20/17 to 20/40 for PHMB 0.02%+ and 20/20 to 20/40 for PHMB 0.08%). Those participants with very poor outcomes of 20/200 or worse were 8 of 61 (13.1%) receiving PHMB 0.02%+ and 9 of 66 participants (13.6%) receiving PHMB 0.08%. Table S9 describes the trial failures, which were similar in both treatment arms: 7 of 61 participants (11.5%) receiving PHMB 0.02%+ and 10 of 66 participants (15.2%) receiving PHMB 0.08% showed similar rates in each arm for the different reasons for failure. Their outcomes after the trial also are summarized in Table S9: the therapeutic keratoplasty rate was 8 of 127 participants (6.3%) overall, 3 of 61 participants (4.9%) receiving PHMB 0.02%+, and 5 of 66 participants (7.5%) receiving PHMB 0.08%. Quality-of-life scores are summarized in Table S10.

**Other Outcome Measures of Interest (Not Prespecified).** These are the Kaplan-Meier crude time-to-eventual-cure analysis (Fig 3A) and the adjusted analysis (Fig 3B). The adjusted analysis results are almost identical for both treatments, with an overall median time to cure of 124 days (4.1 months). In addition, analysis of cure rates for patients with different baseline AK disease stages (Table S11, available at www.aaojournal.org) shows no significant interaction between disease stage and treatment ($P = 0.559$) and no difference in cure rates between stages or treatments. Evaluation of the cure rate (MCR within 12 months) by diagnostic category is provided in Table S12A (available at www.aaojournal.org) for those whose diagnoses were determined by IVCM alone (54/59 participants [91.5%]) compared with those with positive microbiological findings (56/68 participants [82.4%]) and shows no meaningful difference ($P = 0.191$). Nor were meaningful differences found in the proportions of

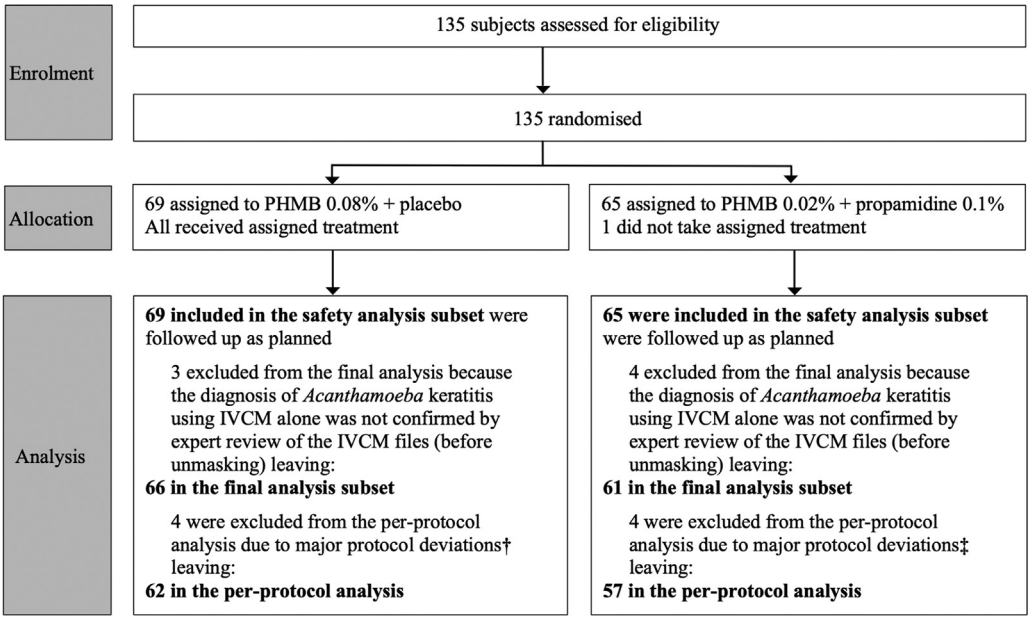



**Figure 2.** Consolidated Standards of Reporting Trials flow diagram for the prospective, randomized, double-masked, active-controlled, multicenter *Acanthamoeba* keratitis phase 3 trial. †One participant received a disallowed medication (self-prescribed over-the-counter propamidine) before randomization, 1 participant did not undergo repeated culture analysis before restarting a course of intensive treatment, and 2 participants did not comply with treatment. ‡One participant received a disallowed medication (self-prescribed over-the-counter propamidine) before randomization, 1 participant was prescribed an antiviral after trial drug discontinuation, but before the end of the study visit, and 2 participants did not comply with treatment. IVCM = in vivo confocal microscopy.

participants with different AK disease stages at baseline as diagnosed by IVCM or microbiological findings (Table S12B, available at www.aaojournal.org). Images showing examples of the disease course, their outcomes, details of treatment, and time to cure for participants with different AK stages at baseline are shown in Figure S4 (available at www.aaojournal.org). Note that some patients were clinically worse at 30 days from baseline and others had not improved, but all were cured medically.

**Safety Outcome Measures.** Adverse events for the safety analysis subset are summarized in Table S13 (available at www.aaojournal.org) and were similar for both treatments. Adverse event details are given in Table S14 (available at www.aaojournal.org). Three of the 13 severe adverse events were the result of presumed toxicity (preferred term, *eye pain*), leading to 3 treatment failures (Table S9) requiring an AAT change, for 2 patients receiving PHMB 0.02%+ and for 1 patient receiving PHMB 0.08%. Secondary safety outcomes in the full analysis subset are listed in Table S15 (available at www.aaojournal.org) and were not clinically or statistically different between treatments.

**Outcomes for Participants Excluded from the Full Analysis Subset.** These were because of an unconfirmed IVCM diagnosis of AK are given in Table S16 (available at www.aaojournal.org). One of these received a diagnosis of concurrent herpes simplex virus 1 week after baseline and discontinued from the study. The others achieved good outcomes with recovery of normal vision.

## Discussion

The medical cure rate of more than 86% (adjusted) for either treatment is one of the best reported since 2000 and is better than those reported in the other comparable studies (Appendices 1A and 1E),[1,2,10] supporting our hypothesis that PHMB 0.08% monotherapy is noninferior, although not more effective, than the dual-therapy comparator.

These trial results may owe as much to the effect of the well-defined drug delivery protocol (Fig S1) as to the drugs used. As described in "Sample Size Determination," the estimated MCR within 12 months observed in the Milan and London retrospective study for the phase 3 comparator was 57 of 85 participants (67%), compared with the phase 3 MCR within 12 months of 54 of 61 participants (88.52%) for PHMB 0.02%+, of whom 47 of 61 participants also were treated in Milan and London. This improvement in outcomes for the comparator was unexpected and was not explained fully by differences in the study centers, the potential effects of the different diamidines used, or baseline risk factors, including AK severity. A principal difference between these studies is

Table 1. Baseline Demographics and Clinical Status of 127 Participants in the Full-Analysis Subset of the Phase 3 Trial

| Baseline Characteristics (Full-Analysis Subset) | Treatment Group | | All Participants |
|---|---|---|---|
| | *Polihexanide 0.02%+ Propamidine* | *Polihexanide 0.08%+ Placebo* | |
| Total no. of participants | 61 | 66 | 127 |
| Demographic characteristics | | | |
| Age (yrs) | | | |
| Mean ± SD | 38.3 ± 14.4 | 35.2 ± 13.2 | 36.7 ± 13.8 |
| Median (interquartile range) | 37 (26–49) | 33.5 (25–44) | 35 (25–47) |
| Minimum–maximum | 17–71 | 15–73 | 15–73 |
| Age group (yrs)* | | | |
| 15–35 | 28 (45.9) | 36 (54.6) | 64 (50.4) |
| 36–73 | 33 (54.1) | 30 (45.5) | 63 (49.6) |
| Sex | | | |
| Female | 35 (57.4) | 39 (59.1) | 74 (58.3) |
| Male | 26 (42.6) | 27 (40.9) | 53 (41.7) |
| BCVA before AK† | | | |
| ≥ 6/6 | 49 (92.5) | 59 (96.7) | 108 (94.7) |
| < 6/6–6/12 | 3 (5.7) | 1 (1.6) | 4 (3.5) |
| 6/15–6/30 | 1 (1.9) | 1 (1.6) | 2 (1.8) |
| Unknown | 8 | 5 | 13 |
| Study center code* | | | |
| 11 Moorfields Eye Hospital, United Kingdom | 30 (49.2) | 28 (42.4) | 58 (45.7) |
| 12 Manchester Eye Hospital, United Kingdom | 1 (1.6) | 1 (1.5) | 2 (1.6) |
| 13 University Hospital Southampton, United Kingdom | 3 (4.9) | 1 (1.5) | 4 (3.1) |
| 21 Ospedale San Raffaele, Milano, Italy | 17 (27.9) | 23 (34.8) | 40 (31.5) |
| 22 Ospedale SS Giovanni e Paolo, Venice, Italy | 5 (8.2) | 9 (13.6) | 14 (11.0) |
| 31 Medical University of Silesia, Katowice, Poland | 5 (8.2) | 4 (6.1) | 9 (7.0) |
| Risk factors for keratitis | | | |
| CL wear | | | |
| No | 3 (4.9) | 2 (3.0) | 5 (3.9) |
| Yes | 58 (95.1) | 64 (97.0) | 122 (96.1) |
| Ocular trauma | | | |
| No | 59 (96.7) | 63 (95.4) | 122 (96.1) |
| Yes | 2 (3.3) | 3 (4.6) | 5 (3.9) |
| Clinical status at baseline | | | |
| Refractive error at baseline‡ | | | |
| Myopia | 49 (81.7) | 55 (85.9) | 104 (83.9) |
| Hyperopia | 7 (11.7) | 8 (12.5) | 15 (12.1) |
| Emmetropia | 4 (6.7) | 1 (1.6) | 5 (4.0) |
| Unknown | 1 | 2 | 3 |
| Time from onset of symptoms (days) | | | |
| Mean ± SD | 36.9 ± 55.0 | 33.5 ± 39.2 | 35.2 ± 37.5 |
| Median (interquartile range) | 20 (8–37) | 19 (8–42) | 20 (8–41) |
| Days from onset of symptoms, grouped* | | | |
| ≤ 41 (quartile 3) | 47 (77.0) | 49 (74.2) | 96 (75.6) |
| > 41 | 14 (23.0) | 17 (25.8) | 31 (24.4) |
| Time from first treatment for keratitis (days) | | | |
| Mean ± SD | 13.5 ± 22.3 | 12.0 ± 22.2 | 12.7 ± 22.2 |
| Median (interquartile range) | 4 (0–20) | 4 (0–17) | 4 (0–18) |
| Antibiotics for keratitis before baseline* | | | |
| No | 11 (18.0) | 10 (15.2) | 21 (16.5) |
| Yes | 50 (82.0) | 56 (84.8) | 106 (83.5 |
| Antivirals for keratitis before baseline* | | | |
| No | 42 (68.9) | 48 (72.7) | 90 (70.9) |
| Yes | 19 (31.1) | 18 (27.3) | 37 (29.1) |
| Antifungals for keratitis before baseline | | | |
| No | 61 (100) | 65 (98.5) | 126 (99.2) |
| Yes | 0 | 1 (1.5) | 1 (0.8) |
| Corticosteroids before baseline (any cause)* | | | |
| No | 41 (67.2) | 35 (53.0) | 76 (59.8) |
| Yes | 20 (32.8) | 31 (47.0) | 51 (40.2) |
| Disease stage at baseline* | | | |
| I | 8 (13.1) | 14 (21.2) | 22 (17.3) |
| II | 46 (75.4) | 41 (62.1) | 87 (68.5) |
| III | 7 (11.5) | 11 (16.7) | 18 (14.2) |

Table 1. (Continued.)

| Baseline Characteristics (Full-Analysis Subset) | Treatment Group | | All Participants |
|---|---|---|---|
| | *Polihexanide 0.02%+ Propamidine* | *Polihexanide 0.08%+ Placebo* | |
| Severe inflammatory disease: scleritis or hypopyon | 0 | 0 | 0 |
| Bilateral disease | | | |
| Absent | 57 (93.4) | 55 (84.6) | 112 (88.9) |
| Present | 4 (6.6) | 10 (15.4) | 14 (11.1) |
| Unknown | 0 | 1 | 1 |

AK = *Acanthamoeba* keratitis; BCVA = best-corrected visual acuity; CL = contact lens; SD = standard deviation.
Data are presented as no. (%), unless otherwise specified in the row. Percentages are of valid totals and exclude missing values.
*Covariates included in the adjusted analysis of the main outcome medical cure rate at 12 months.
†The BCVA before AK was obtained from history, optometry, or hospital records. For those without a past history of eye disease, when written records were not available, the acuity was recorded as 20/20.
‡The refractive error at baseline was obtained from the participant's spectacle or CL prescription data or from optometry or hospital records.

that, in the retrospective study, the drugs were delivered using varied protocols, determined by individual physician's standards of care, as opposed to the detailed drug delivery protocol adopted for the phase 3 trial which may have accounted for the unanticipated improvement in outcomes. Some of the key phase 3 protocol requirements (Fig S1) included specified intensity and frequency of AAT for the initial 19-day intensive phase (also used for relapses of infection), followed by a specified maintenance frequency and specified protocol for discontinuing therapy after meeting a defined standard for a clinical cure. The management of adjunctive topical corticosteroid therapy also was specified with guidance on how this should be withdrawn in participants using these at baseline and an embargo on their use before completing 3 weeks of AAT treatment in those not using steroids at baseline, after which it could be introduced at the investigator's discretion. The protocol for steroid withdrawal also was specified to be at the point at which the criteria for a clinical cure had been met, with steroid therapy having to be discontinued 30 days before the discontinuation of AAT. This protocol is much more detailed than those reported in the 2 randomized controlled trials and 6 prospective nonrandomized case series summarized in Appendix 1F and, to our knowledge, is the only published protocol meeting the criteria for the evaluation of an evidence-based empirically supported

Table 3. Diagnostic Test Results for 127 Participants in the Full-Analysis Subset of the Phase 3 Trial

| Diagnostic Test Results, Full-Analysis Subset | Treatment Group | | All Participants |
|---|---|---|---|
| | *PHMB 0.02%+ Propamidine* | *PHMB 0.08%+ Placebo* | |
| IVCM results | | | |
| Positive | 61 (100.0) | 66 (100.0) | 127 (100.0) |
| Negative | 0 | 0 | 0 |
| Unknown | 0 | 0 | 0 |
| Total | 61 | 66 | 127 |
| Culture results | | | |
| Positive | 17 (28.8) | 19 (30.2) | 36 (29.5) |
| Negative | 42 (71.2) | 44 (69.8) | 86 (70.5) |
| Unknown | 2 | 3 | 5 |
| Total | 61 | 66 | 127 |
| PCR results | | | |
| Positive | 25 (49.0) | 33 (55.0) | 58 (52.3) |
| Negative | 26 (51.0) | 27 (45.0) | 53 (47.7) |
| Unknown | 10 | 6 | 16 |
| Total | 61 | 66 | 127 |
| Test results as basis for diagnosis of AK | | | |
| IVCM positive only | 29 (47.5) | 30 (45.5) | 59 (46.5) |
| IVCM and culture positive, PCR negative or unreported | 7 (11.7) | 3 (4.5) | 10 (7.9) |
| IVCM and PCR positive, culture negative or unreported | 15 (25.0) | 17 (25.8) | 32 (25.4) |
| IVCM, culture, and PCR positive | 10 (16.7) | 16 (24.2) | 26 (20.6) |
| Unknown | 0 | 0 | 0 |
| Totals | 61 | 66 | 127 |

AK = *Acanthamoeba* keratitis; IVCM = in vivo confocal microscopy; PCR = polymerase chain reaction.
Data are presented as no. (%), unless otherwise specified. Percentages are of valid totals and exclude missing values. In addition to IVCM, culture and PCR analyses were carried out on all participants. Because no validated PCR analysis is available in Europe or the United Kingdom, the test was carried out by the local providers for each center.

Table 4. Primary Outcome for a Medical Cure (without Surgery) at 12 Months for the Full-Analysis Subset with and without Adjustment for Imbalance in Baseline Risk Factors

| Treatment Group | Primary Outcome for the Full-Analysis Set* | | | | | |
|---|---|---|---|---|---|---|
| | Unadjusted Results | | | | Adjusted for Confounding† | |
| | Number | Medical Cure | Medical Cure Rate (%) | Binomial Exact 95% CI for Proportion Resolved | Adjusted Efficacy (%) | 95% CI for the Proportion Resolved |
| PHMB 0.02%+ propamidine 0.1% | 61 | 54 | 88.52 | 77.78–95.26 | 86.55 | 79.06–94.04 |
| PHMB 0.08%+ placebo | 66 | 56 | 84.85 | 73.90–92.49 | 86.68 | 79.54–93.81 |
| Total | 127 | 110 | 86.61 | 79.4–92.0 | 86.61 | 81.29–91.94 |
| Medical cure rate within 12 months in the 2 arms | | | Exact $P = 0.609$ | | Exact $P = 0.980$ | |

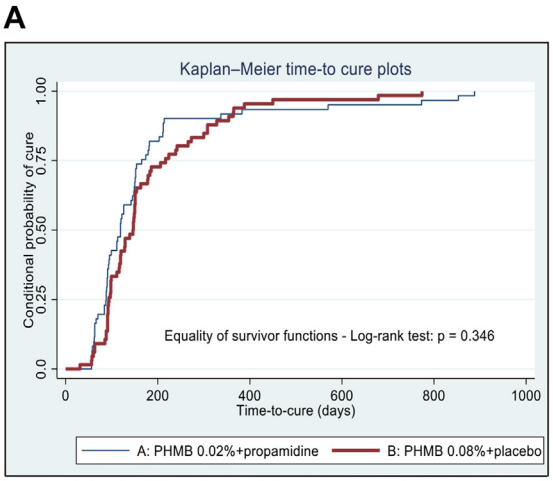
AAT = antiamebic therapy; CI = confidence interval.
*Primary outcome: clinical resolution within 12 mos of starting AAT, without surgery, and without a relapse within 30 days of stopping AAT (checked at 90 days).
†Covariates (n = 7) adjusted for (Table 1) were: age (binary, based on median), AK stage (1, 2, or 3), delay in diagnosis in days from onset of symptoms to baseline (binary, based on 75th percentile), corticosteroid use before baseline (no or yes), antibiotic use before baseline (no or yes), antiviral drug use before baseline (no or yes), and study site (6 coded categories).

treatment protocol[11] for AK having been developed with a theoretical rationale and evaluated empirically in the context of a methodologically rigorous randomized controlled trial at more than 1 site, by more than 1 investigator, with real patients, and with rigorous and masked evaluation of outcomes.[11] As a result, we cannot be sure that the results we have described can be achieved using a different drug delivery protocol. Also notable is the time taken for some trial participants to respond to treatment of more than 30 days: 7 of the examples shown in Figure S4, all of whom were medically cured. Two previous AK treatment trial protocols have specified improvement at 2 weeks as a primary outcome measure

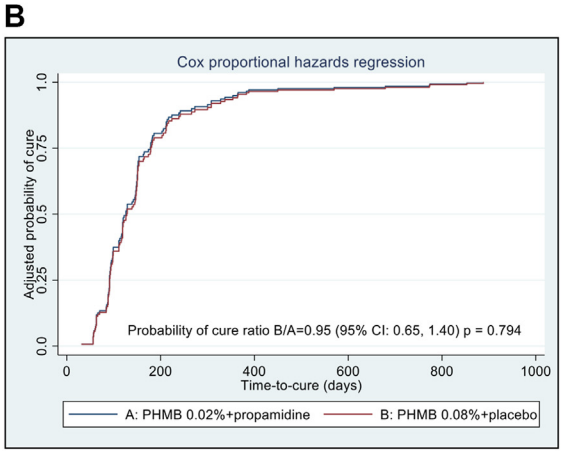
Table 7. Secondary Outcome Measures

| Secondary Outcomes | Treatment Group (Full-Analysis Subset) | | All Participants (n = 127) |
|---|---|---|---|
| | PHMB 0.02%+ Propamidine (n = 61) | PHMB 0.08%+ Placebo (n = 66) | |
| Best-corrected visual acuity summary* | | | |
| Mean (Snellen feet) | 20/40 +1 | 20/40 | 20/40 +1 |
| Median (IQR) | 20/20 (20/25 +1 to 20/40) | 20/20 (20/20–20/40) | 20/20 (20/20–20/40) |
| Corneal scarring | | | |
| Baseline | 3 (4.9) | 0 | 3 (2.4) |
| End of study | | | |
| Present | 30 (54.5) | 33 (51.6) | 63 (52.9) |
| Absent | 25 (45.6) | 31 (48.4) | 56 (47.1) |
| Missing data, no. | 6 | 2 | 8 |
| Trial failure (trial treatment stopped for all)† | 7 (11.5) | 10 (15.2) | 17 (13.4) |
| Primary reasons for withdrawal | | | |
| Presumed toxicity | 2 (3.3) | 1 (1.5) | 3 (2.4) |
| Failure to improve | 4 (6.6) | 6 (9.1) | 10 (7.8) |
| Corneal perforation | 1 (1.6) | 0 | 1 (0.8) |
| Still receiving treatment after 12 mos | 0 | 1 (1.5) | 1 (0.8) |
| Concurrent herpes simplex keratitis developed | 0 | 1 (1.5) | 1 (0.8) |
| Lost to follow-up before data locked, but medically cured within 12 mos | 0 | 1 (1.5) | 1 (0.8) |
| Total | 7 | 10 | 17 |
| Quality-of-life score change from baseline to end of study, median (IQR)‡ | | | |
| EQ-5D-5L VAS score§ | 15 (5–30) | 14.5 (5–28) | Not reported |
| VFQ-25 composite score | 21.4 (7.7–35.0) | 22.1 (7.9–37.3) | Not reported |

IQR = interquartile range; VAS = visual analog scale; EQ-5D-5L = the EuroQol 5 Dimension 5 Level health related quality of life questionnaire; VFQ-25 = the 25 Item Visual Function Questionnaire.
Data are presented as no. (%), unless otherwise indicated. Because no clinically or statistically significant differences were found between treatments, no *P* values or confidence intervals were added.
*Best-corrected visual acuity outcomes in full given in Supplemental Table 8.
†Outcomes for treatment failures are given in detail in Supplemental Table 9; definition of failure is given in "Methods."
‡Full details of quality-of-life tool scores are given in Supplemental Table 10.
§Scored between 0 and 100 for current overall health-related quality of life.




| Time to cure | PHMB 0.02% + propamidine | PHMB 0.08% + placebo | All |
|---|---|---|---|
| Median | 119 | 146.5 | 126 |
| Q1, Q3† | 88, 165 | 94, 217 | 91, 182 |




| Adjusted time to cure | PHMB 0.02% + propamidine | PHMB 0.08% + placebo | All |
|---|---|---|---|
| Median | 122 | 126 | 124 |
| Q1, Q3† | 90, 175 | 91. 177 | 91, 176 |

**Figure 3.** Kaplan–Meier plots showing time-to-eventual-cure analysis for the full analysis subset. This includes all the trial failures at the point of a cure of *Acanthamoeba* infection as defined by discontinuation of antiamebic treatment (AAT). This analysis differed from the analysis described in the phase 3 trial statistical plan, which was the time to cure for all participants defined as cured or not cured at the 30-day follow-up (checked at 90 days) either after discontinuation of trial drugs or within 12 months of starting AAT, whichever came first. Treatment A is PHMB 0.02%+ 0.1% and treatment B is PHMB 0.08% with placebo. **A**, Conditional probabilities of cure with crude estimates unadjusted for confounders in the phase 3 trial for the full-analysis subset (N = 127). **B**, Estimated probabilities of cure adjusted for baseline covariates in the phase 3 trial full-analysis subset (N = 127). Baseline covariates were adjusted for: age (1 or 2, based on median), *Acanthamoeba* keratitis stage (1, 2, and 3), days from start of symptoms to AAT (1 or 2, based on quartile 3), prior topical corticosteroids (no or yes), prior antibiotics (no or yes), prior antivirals (no or yes), and trial center. †Interquartile range. Q = quartile.

(Appendix 1F), which our study suggests may be too early to predict treatment failure.

For PHMB 0.08% to be used as monotherapy based on our results, the potential effect of the BAC 0.005%[3,12] included in the placebo must also be taken into account, which may have added to the antiamebic activity of PHMB 0.08% given with placebo in this trial. The size of the effect of BAC 0.005% can be estimated from an in vitro study[12] on the minimum cysticidal concentrations of drugs showing that, compared with PHMB, the minimum cysticidal concentration was 32-fold less for Brolene and 40-fold less for BAC 0.005% combined with levofloxacin. These data suggest that BAC 0.005% alone is unlikely to have a significant effect on the outcome of PHMB 0.08% given as monotherapy. Furthermore, if placebo was instilled at the same time as PHMB 0.08%, instead of 5 minutes before as directed, this may have diluted the PHMB, reducing its effect; this is a compliance issue for dual therapies and one reason why monotherapy is preferable if shown to be as effective. We conclude that PHMB 0.08% monotherapy is noninferior to PHMB 0.02%+ propamidine 0.1% dual therapy and is a better choice for first-line treatment of AK both when the protocol used for this trial is followed and when using the eye drops manufactured for the trial to GMP quality standards. Neither treatment resulted in significant toxicity in the relatively small numbers treated in this trial.

The trial has limitations. In vivo confocal microscopy was prespecified as the inclusion criterion, knowing that reliance on PCR or culture analysis would have excluded many participants with AK, resulting in a less generalizable study, and would have delayed the start of treatment. However, this approach led to exclusion from the full analysis subset of 7 or 134 participants (5.2%) having an

IVCM diagnosis unsupported by microbiological diagnosis and for whom expert review of the IVCM files did not meet the study criteria for the diagnosis of AK. Diagnosis of AK on clinical grounds is common,[13] and although these participants may or may not have had AK, the keratitis responded well to both treatments (Table S16). The PCR-positive findings rate of 58 of 127 participants (52.3%) in the study is lower than the 70% commonly referenced,[3] although consistent with the findings of 2 institutional United Kingdom studies using general (nonophthalmic) culture and PCR service providers.[13,14] Despite these diagnostic safeguards, it remains possible that some participants without AK were included in the trial. That this is unlikely is shown by the analysis (Table S12) in which no significant differences were found between diagnostic categories for either the overall outcome or the baseline disease severity. However, the inclusion of a few participants without AK in the trial would have had an equal chance of being randomized to each treatment group, which should not result in treatment bias, although it might have enhanced the medical cure rate. It is possible that other centers are faced with a higher proportion of patients having more advanced disease at diagnosis than those included in this trial, of whom 18 of 127 (14.2%) had stage 3 disease (corneal ring infiltrate). Example images of outcomes for these and other disease stages in trial participants are shown in Figure S4. The relatively low number of patients with stage 3 disease in the trial was not the result of the exclusion of advanced disease by the trial protocol but is likely to have resulted from increased awareness of AK as a cause of microbial keratitis by ophthalmologists in the regions using the trial centers; this awareness resulting in early stage diagnosis compared to what is seen in other countries and regions where misdiagnosis of early stage disease is common. Furthermore, the analysis of outcomes for different disease stages at baseline showed no difference in cure rates between stages for either treatment (Table S11), although this is probably because of small numbers. Poorer outcomes than those reported herein might be expected in patients with advanced disease at diagnosis. Finally, measuring PHMB in biological fluids and tissues was an aim of an early Orphan Drug for *Acanthamoeba* Keratitis study, but proved impossible (Appendix 6, available at www.aaojournal.org). As a result, we have no data on the penetration of PHMB 0.08% to support our hypothesis that the higher concentration of PHMB at 0.08% would result in a higher corneal stromal concentration and penetration of PHMB than that achievable with PHMB 0.02%, leading to improved clinical efficacy.

We have shown that PHMB 0.08% (0.8 mg/ml) monotherapy can provide noninferior results to the dual-therapy comparator, providing medical cure rates of more than 86%. These results may only apply when using the GMP quality manufactured eye drops available for this study in populations with AK with similar disease severity and when used with the study protocol. Similar results cannot be expected using different treatment delivery protocols that are not derived from evidence-based outcomes. Successful licensing of PHMB 0.08% can be expected to improve both the quality of PHMB therapy for AK and to reduce treatment delays when PHMB is unavailable off the shelf and must be compounded for an individual patient.

## Footnotes and Disclosures

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HUMAN SUBJECTS: Human subjects were included in this study. The study was designed and monitored and conducted in accordance with good clinical practice guidelines and the Declaration of Helsinki. Ethics approvals (see Appendix S2) were obtained before the start at all trial centers and all participants gave informed consent.

No animal subjects were included in this study.

Author Contributions:

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Analysis and interpretation: Dart, Papa, Hau, Minassian

Data collection: Dart, Papa, Rama, Knutsson, Ahmad, Hau, Sanchez, Franch, Birattari, Leon, Fasolo, Mrukwa Kominek, Jadczyk-Sorek, Carley, Hossain

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Abbreviations and Acronyms:
**AAT** = antiamebic therapy; **AK** = *Acanthamoeba* keratitis; **BAC** = benzalkonium chloride; **GMP** = Good Manufacturing Practice; **IVCM** = in vivo confocal microscopy; **MCR** = medical cure rate; **PCR** = polymerase chain reaction.

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