



Original Research

An artificial tear containing flaxseed oil for treating dry eye disease: A randomized controlled trial



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ARTICLE INFO

Keywords:

Dry eye disease
Omega-3
Fatty acid
Nano-emulsion
Artificial tears
Flaxseed oil
Trehalose

ABSTRACT

Purpose: To evaluate the efficacy and safety of a nano-emulsion artificial tear (OM3) containing carboxymethylcellulose (CMC) and glycerin, flaxseed oil and castor oil, and three osmoprotectants (levocarnitine, erythritol, and trehalose) compared with an artificial tear (Refresh Optive Advanced [ROA]) containing the same ingredients with the exception of trehalose and flaxseed oil.

Methods: In this multicenter, double-masked, randomized, two-arm, parallel-group, 6-visit study (screening, baseline, and days 7, 30, 60, and 90), subjects with dry eye disease underwent an open-label, 7-day run-in with CMC 0.5% (Refresh Plus), before 1:1 randomization to OM3 or ROA for 90 days (both instilled ≥ 2 daily). Ocular Surface Disease Index (OSDI; primary endpoint change from baseline at day 90), tear film breakup time (TBUT), and ocular staining (combined/corneal/conjunctival) were assessed; change from baseline in these parameters was calculated at each timepoint. Treatment-related adverse events (AEs) were assessed at each visit.

Results: Overall, 242 subjects were randomized (OM3, $n = 120$; ROA, $n = 122$). At day 90, significant improvements in OSDI, ocular staining and TBUT were evident in both treatment groups. Significant ($P < 0.05$) between-group differences in favor of OM3 were observed for combined ocular staining (all timepoints), corneal staining (day 90), and conjunctival staining (day 30). Treatment-related AEs were higher in the ROA (9.8%) versus OM3 (6.7%) group; blurred vision was among the most commonly reported AE (OM3 0% vs ROA 4.1%).

Conclusion: These findings support the application of OM3, a novel preservative-free, nano-emulsion tear formulation with trehalose and flaxseed oil, for the treatment of dry eye disease.

1. Introduction

Dry eye is a highly prevalent, multifactorial disease characterized by a loss of tear film homeostasis [1]. Tear instability is a core mechanism in dry eye disease, and tear lipid deficiency, which leads to excessive evaporation, particularly under adverse environmental conditions, is a common contributory factor [2,3]. Evaporative dry eye (characterized by tear lipid abnormality, and associated with a short tear breakup time [TBUT]) and aqueous-deficient disease (characterized by reduced tear secretion) frequently coexist [1]. Up to 50% of the global population experience dry eye symptoms, with the prevalence of signs reported to

be higher but more variable [4]. Symptoms of dry eye disease, such as ocular discomfort and blurred vision, can negatively impact visual function and individuals' quality of life [5–8]. As symptoms commonly derive from insufficiencies of both aqueous and lipid tear film constituents, lipid-containing artificial tears, which supplement both components, can benefit the majority of dry eye patients by reducing evaporation and improving tear film stability [3,9]. Lipid-containing artificial tears usually consist of humectant polymers, such as carboxymethylcellulose (CMC), hyaluronic acid or polyethylene glycol, which promote hydration and lubrication of the ocular surface, along with lipids (eg, castor oil, mineral oil) to assist with maintaining tear lipid

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<https://doi.org/10.1016/j.jtos.2019.11.004>

Received 21 July 2019; Received in revised form 25 October 2019; Accepted 13 November 2019

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layer integrity, and salt (eg, Na^+ or K^+) or organic osmolytes that protect ocular surface cells from hyperosmotic stress [10].

OM3 (Refresh Optive® MEGA-3 Preservative-Free Lubricant Eye Drops, Allergan plc, Dublin, Ireland) is a novel tear formulation containing CMC 0.5%, glycerin 1%, polysorbate 80, emulsified castor oil and flaxseed oil, two organic osmolytes (levocarnitine and erythritol), and trehalose, a disaccharide of glucose. CMC and glycerin are the surface lubricant and humectant. The two organic osmolytes, levocarnitine and erythritol, are also known as compatible solutes. These are small organic molecules that can enter the cells to help balance the osmotic stress without causing cell damage [11,12]. Trehalose is well known for its protein and lipid stabilization effects, and it can protect cells against hyperosmotic stress from the extracellular level [13]. Both organic osmolytes (levocarnitine and erythritol) and trehalose are known as osmoprotectants. Trehalose also serves as an antioxidant, along with butylated hydroxytoluene in the formulation, to stabilize the flaxseed oil. The surfactant polymer and ophthalmic demulcent, polysorbate 80, has lubricating properties and also promotes the formation of the nano-emulsion—in this case, a biphasic dispersion of water-soluble polymers and oil droplets (typically ranging from 20–200 nm), stabilized by an amphiphilic surfactant [14,15].

Oral supplementation with long-chain omega-3 fatty acids (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) has been shown to improve dry eye signs and symptoms, and to provide neuroprotection to corneal nerves in recent studies [16–18], although a recent clinical trial could not corroborate a significant therapeutic effect on dry eye with high-dose, long-chain oral omega-3 fatty acid supplementation relative to placebo (olive oil) [19]. In terms of local ocular delivery of omega-3 fatty acids, a recent trial considered the potential benefit of an eye drop preparation containing long-chain omega-3 fatty acids for the treatment of contact lens discomfort; relative reductions in tear film inflammatory cytokines, but no significant effect on ocular comfort, were reported relative to placebo [20]. However, the effectiveness of topical omega-3 fatty acids in artificial tear formulations has not yet been evaluated for treating dry eye disease.

The purpose of this study was to evaluate the safety, efficacy, tolerability, and acceptability of the OM3 nano-emulsion formulation, in comparison to a marketed emulsion artificial tear containing CMC 0.5%, glycerin 1%, emulsified castor oil (with polysorbate 80), levocarnitine, and erythritol (Refresh Optive® Advanced Preservative-Free Lubricant Eye Drops [ROA], Allergan plc).

2. Subjects and methods

2.1. Study design

This multicenter, double-masked, randomized, two-arm, parallel-group study was conducted in the United States (10 sites) and Australia (3 sites) (ClinicalTrials.gov Identifier: [NCT02553772](https://clinicaltrials.gov/ct2/show/study?term=NCT02553772)). The study was conducted in compliance with Good Clinical Practice, the International Conference on Harmonization Guidelines, and the ethical principles of the Declaration of Helsinki. The protocol received approval by an institutional review board or human research ethics committee at each site prior to study initiation. All subjects provided written informed consent before initiating any study procedures.

2.1.2. Key eligibility criteria

Subjects were enrolled if they satisfied the following criteria: aged ≥ 18 years with previous use of artificial tears for dry eye disease; an Ocular Surface Disease Index (OSDI) score of ≥ 28 and ≤ 65 at screening (day -7) and ≥ 18 and ≤ 65 at baseline (day 1); three consecutive TBUT tests ≤ 10 seconds in at least 1 eye at screening; grade 1 to 4 (modified National Eye Institute [NEI] Grid; score range, 0 to 5) staining in at least 1 of 5 areas of the cornea or 1 of 6 areas of the conjunctiva in at least 1 eye at screening and baseline; and currently

corrected distance visual acuity of at least 20/32 Snellen equivalent in each eye (3-meter logMAR chart) with habitual spectacle correction (if necessary) at screening.

Key exclusion criteria were the following: Schirmer test (with anesthesia) ≤ 2 mm in either eye at screening; corneal or conjunctival staining score of 5 in any area of either eye at screening or baseline; total corneal staining score of > 18 at screening or baseline; use of any systemic medication that may affect tear film or vision for < 3 months prior to screening or a change in dosage anticipated during the study; ocular/ophthalmic surgery or trauma, which could affect corneal sensitivity and/or tear distribution within 6 months prior to screening; current or previous use of topical ocular medication within 2 weeks of screening (except cyclosporine ophthalmic emulsion 0.5% [Restasis®, Allergan plc] and intraocular pressure-lowering agents for glaucoma if they had been used by subjects for < 3 months prior to screening); and use of contact lens anticipated during the study or use ≤ 3 months prior to screening.

2.1.3. Study treatment

The study involved a total of 6 visits: screening (days -10 to -7), baseline (day 1), day 7 (± 3 days), day 30 (± 7 days), day 60 (± 7 days), and day 90 (± 7 days). Following an initial open-label, 7-day run-in period using CMC 0.5% (Refresh Plus® Preservative-Free Lubricant Eye Drops; Allergan plc), at baseline (day 1), enrolled subjects were randomized 1:1 in a double-masked fashion to receive either OM3 or ROA for 90 days (Fig. 1). ROA consists of the same active ingredients as OM3, but does not contain flaxseed oil and trehalose. The randomization scheme was prepared by the sponsor's biostatistics group and managed using an interactive response technology system; the treatment allocation(s) were concealed from the site investigators and study subjects. Randomization was stratified by baseline OSDI score (mild/moderate, ≥ 18 to ≤ 32 ; severe, > 32 to ≤ 65). Subjects were instructed to instill 1 to 2 drops of the assigned treatment in each eye, as needed, but at least twice daily. To preserve double-masking, all products were supplied by the study sponsor in identical 0.4-mL unit dose vials.

2.1.4. Concurrent medications

After day -7 (screening), subjects were instructed not to begin or stop the use of any systemic medications (including over-the-counter, herbal, or prescription medications, or nutritional supplements) that may affect a dry eye condition or vision (eg, cause blurred vision, cataracts, or glaucoma).

2.1.5. Outcome measures

The primary efficacy endpoint was the change from baseline in OSDI total score at day 90 in the intent-to-treat (ITT) population (all randomized subjects). The OSDI is a validated 12-item survey that assesses the severity of dry eye symptoms based on a score from 0 (no disease) to 100 (maximum severity of disease) [21].

The secondary efficacy endpoints included change from baseline at each follow-up visit in the ITT population in OSDI (excluding day 90), TBUT (with fluorescein, measured 3 times in each eye), corneal fluorescein staining score (modified NEI Grid in 5 zones of the cornea, using a fluorescein strip moistened with 1 drop of preservative-free saline with 2 minutes of waiting until grading; maximum score of 25 per eye), conjunctival lissamine green staining score (modified NEI Grid in 6 zones of the conjunctiva, using a lissamine green strip moistened with 1 drop of preservative-free saline with 10 seconds of waiting until grading; maximum score of 30 per eye) [22,23], and combined ocular staining (summing the corneal and conjunctival staining scores; maximum score of 55 per eye). Changes from baseline in OSDI scores were also presented by baseline OSDI category: mild/moderate dry eye

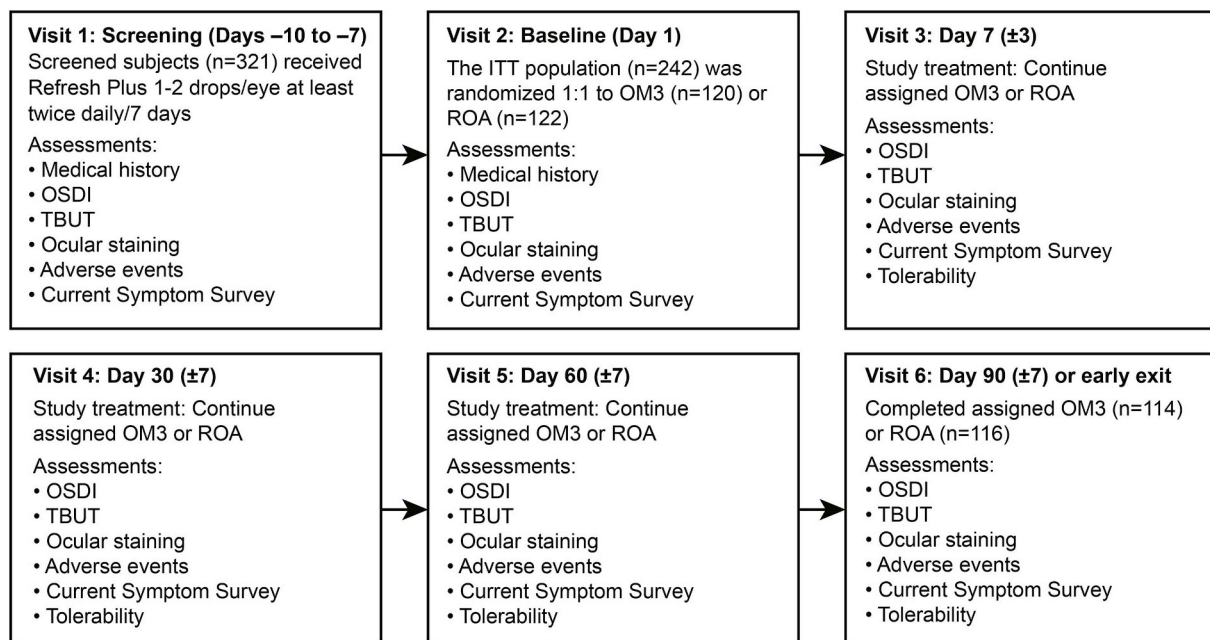


Fig. 1. Study design, schedule of key assessments, and subject flow through the clinical trial. ITT, intent-to-treat; OM3, Refresh Optive MEGA-3 Preservative-Free Lubricant Eye Drops; OSDI, Ocular Surface Disease Index; ROA, Refresh Optive Advanced Preservative-Free Lubricant Eye Drops; TBUT, tear breakup time.

(OSDI score 18–32 at baseline) and severe dry eye (OSDI score 33–65 at baseline). Safety was assessed at each follow-up visit and included adverse event (AE) monitoring and slit-lamp biomicroscopy examination.

Other secondary efficacy endpoints included subject-reported outcomes measured using a visual analog scale (VAS), ranging from 0 to 100. The Current Symptom Survey evaluated five dry eye symptoms (burning/stinging; grittiness/foreign body sensation; dryness; blurry/fluctuating vision; overall ocular pain/discomfort) over the previous 24 hour (on a scale from no symptoms = 0 to symptoms of maximum intensity = 100) and was administered at all study visits. In the 8-question Tolerability Questionnaire, subjects were instructed to mark a vertical line on the VAS to best describe how they felt over the prior week during the first 30 minutes after study drop instillation at each follow-up visit. The 8 questions included 4 positive rating questions (comfortable, soothing, moistening/lubricating, and clear vision) and 4 negative rating questions (stickiness, blur, burning/stinging, and discomfort).

2.1.6. Statistical analysis

The primary efficacy analysis was performed for the ITT population using combined noninferiority and superiority tests [24], with the last observation carried forward to impute missing data. In order to control the overall Type I error of multiple testing of the primary efficacy analysis, the significance level was adjusted by applying a gatekeeping procedure. If the upper limit of the two-sided 95% confidence interval (CI) based on the treatment difference in OSDI score (OM3 minus ROA) was less than 7.3 units, then the OM3 formulation was considered noninferior to ROA [25], and a two-sided test for superiority was performed at the 0.05 significance level. Both noninferiority and superiority tests used an analysis of variance (ANOVA) model, with treatment and OSDI score stratum as fixed effects.

The same ANOVA model was used to assess changes from baseline in secondary efficacy endpoints. Within-group changes from baseline were analyzed using the paired *t* test (excluding OSDI at all other visits). Unless otherwise specified, only observed values were used. Missing data were not imputed. *P*-values, treatment differences, and 95% confidence intervals (CIs) for between-group comparisons were obtained

from the same ANOVA model.

Treatment-emergent adverse events (AEs) and biomicroscopy findings were summarized using descriptive statistics. For the subject-reported outcomes, descriptive statistics were provided for each item at each visit by treatment group. Within-group changes from baseline in the Current Symptom Survey were analyzed using paired *t* tests.

Post hoc efficacy analyses were performed in order to understand how the study treatments performed in sub-populations with different severities of dry eye signs and symptoms at baseline. Based on the mean ($\pm$ standard deviation [SD]) change from baseline at each visit, the following parameters were evaluated: corneal and conjunctival combined staining in subjects with a combined staining score of < 14 (mild/moderate) versus ≥ 14 (clinically relevant) [23] and OSDI scores ≤ 32 (mild/moderate) and > 32 (severe) [21]; corneal and conjunctival staining in subjects stratified by mild/moderate (≤ 32 OSDI score) and severe (> 32 OSDI score) dry eye symptoms [21] at baseline; corneal staining in subjects with a baseline corneal staining score of < 7 (mild/moderate) versus ≥ 7 (clinically relevant) [23]. An additional post hoc analysis was conducted for OSDI scores, TBUT, and ocular surface staining in subjects with short TBUT at baseline (≤ 5 seconds) [26,27]. A post hoc analysis was also performed assessing the average of the scores for the five dry eye symptoms comprising the Current Symptom Survey.

Based on a one-sided Type I error rate of 0.025 and assumptions of no inherent treatment difference and a common SD of 16.319 derived in a previous study [23], 109 subjects per treatment group were determined to be required to achieve 91% power for a one-sided noninferiority test for a between-group difference (ie, the noninferiority margin) of 7.3 units in the mean change from baseline in the total OSDI score at day 90. With a 1:1 treatment allocation and estimated 10% dropout rate, a total of 242 subjects were to be enrolled for 218 subjects to complete the study at day 90.

3.1. Results

3.1.1. Demographics and baseline characteristics

All 242 randomized subjects were included in both the ITT and safety populations (OM3, $n = 120$; ROA, $n = 122$). A total of 12

Table 1
Subject demographic and disease characteristics at baseline (ITT population).

Characteristic	OM3 (n = 120)	ROA (n = 122)
Age, years, mean $\pm$ SD	54.3 $\pm$ 17.3	52.8 $\pm$ 16.7
Female, n (%)	84 (70.0)	95 (77.9)
Race, n (%) ^a		
White	103 (85.8)	99 (81.1)
Non-white	17 (14.2)	22 (18.0)
OSDI score, mean $\pm$ SD	41.1 $\pm$ 12.7	39.6 $\pm$ 11.3
OSDI category [†] , n (%)		
Mild/moderate (18–32)	34 (28.3)	34 (27.9)
Severe (33–65)	86 (71.7)	88 (72.1)
TBUT (s), mean $\pm$ SD	4.8 $\pm$ 1.7	4.9 $\pm$ 1.7
Corneal staining (sodium fluorescein, NEI grade), mean $\pm$ SD	4.5 $\pm$ 3.4	4.5 $\pm$ 3.3
Conjunctival staining (lissamine green, NEI grade), mean $\pm$ SD	6.7 $\pm$ 5.6	5.9 $\pm$ 4.5
Combined ocular surface staining score, mean $\pm$ SD	11.0 $\pm$ 8.0	10.1 $\pm$ 6.6
Schirmer test, mm/5 min, mean $\pm$ SD	9.9 $\pm$ 7.1	9.9 $\pm$ 6.8

ITT, intent-to-treat; NEI, National Eye Institute; OM3, Refresh Optive MEGA-3 Preservative-Free Lubricant Eye Drops; OSDI, Ocular Surface Disease Index; ROA, Refresh Optive Advanced Preservative-Free Lubricant Eye Drops; SD, standard deviation; TBUT, tear breakup time.

^aData missing for one subject (0.8%) in the ROA group.

[†]OSDI score ranges from 0 to 100.

subjects withdrew from the study, of which 5 withdrawals were due to AEs: 2 in the OM3 group (fall and meniscus injury $n = 1$, chalazion, $n = 1$) and 3 in the ROA group (herpes zoster $n = 1$, eye irritation $n = 1$, blurred vision/eye irritation $n = 1$). There were no statistically significant differences between treatment groups with respect to baseline characteristics; the majority of subjects (71.9%) had a severe OSDI score at baseline (Table 1).

3.1.2. OSDI: primary and secondary outcomes

Statistically significant ($P < 0.001$) improvements in OSDI score were observed at day 90, relative to baseline, in both the OM3 (mean $\pm$ SD: 15.6 $\pm$ 16.5) and ROA groups (-13.2 ± 17.7), with a mean (95% CI) treatment difference (OM3 – ROA) of -2.5 ($-6.7, 1.7$). The upper limit of the 95% CI was below the prespecified clinical margin of 7.3 units, indicating that the OM3 formulation was statistically noninferior to ROA.

OSDI scores were significantly reduced from baseline at the earlier time points (days 7, 30, 60) in both treatment groups ($P < 0.001$), but the between-group treatment differences were not statistically significant (Fig. 2; $P > 0.05$). No statistically significant between-group

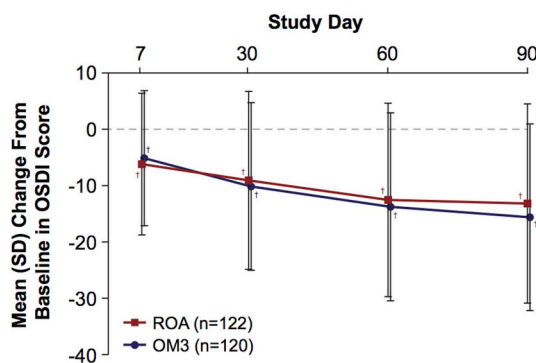


Fig. 2. Mean ($\pm$ SD) change from baseline in Ocular Surface Disease Index (OSDI) score at each study visit (intent-to-treat population). $\dagger P < 0.05$, within-group differences. OM3, Refresh Optive MEGA-3 Preservative-Free Lubricant Eye Drops; ROA, Refresh Optive Advanced Preservative-Free Lubricant Eye Drops. SD, standard deviation.

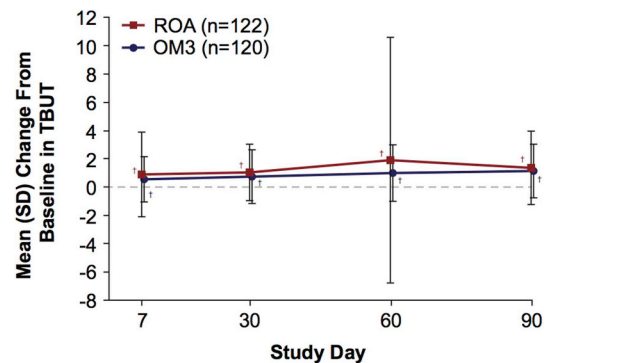


Fig. 3. Mean ($\pm$ SD) change from baseline in tear breakup time (TBUT; seconds) at each study visit (intent-to-treat population). OM3, Refresh Optive MEGA-3 Preservative-Free Lubricant Eye Drops; ROA, Refresh Optive Advanced Preservative-Free Lubricant Eye Drops; SD, standard deviation. $\dagger P < 0.05$, within-group differences.

treatment differences were evident when subjects were stratified by baseline OSDI (mild/moderate or severe disease) in the subgroup analyses.

3.1.3. TBUT and ocular surface staining

At day 90, both treatment groups showed significant ($P < 0.001$) improvements in TBUT from baseline, with a mean $\pm$ SD change of 1.1 ± 1.9 and 1.3 ± 2.6 s for the OM3 and ROA groups, respectively. Significant improvements from baseline were also observed at days 7, 30, and 60 in both treatment groups ($P \leq 0.022$ for both groups), but the between-group differences were not significant at any time point (Fig. 3).

Statistically significant improvements from baseline in the combined corneal and conjunctival staining score were observed at each follow-up visit in the OM3 group ($P \leq 0.010$) and at only day 90 in the ROA group ($P = 0.006$), with statistically significant ($P \leq 0.030$) differences in favor of OM3 for the between-group comparison at each follow-up visit (Fig. 4A). At day 90, the mean $\pm$ SD change from baseline in combined scores was -3.0 ± 5.3 for the OM3 group and -1.4 ± 5.6 for the ROA group; a mean (95% CI) treatment difference of -1.59 ($-2.97, -0.22$; $P = 0.024$).

When corneal and conjunctival staining scores were analyzed separately, statistically significant improvements from baseline were observed at each follow-up visit in the OM3 group ($P \leq 0.003$ for corneal and $P \leq 0.050$ for conjunctival scores) and at day 90 only in the ROA group ($P = 0.021$ for corneal and $P = 0.002$ for conjunctival scores). Additionally, significant between-group differences in favor of OM3 were observed for the change from baseline in mean $\pm$ SD corneal staining at day 90 (-1.5 ± 2.4 vs -0.6 ± 2.7 ; mean [95% CI] difference -0.91 [$-1.57, -0.26$]; $P = 0.007$) and conjunctival staining at day 30 (-0.85 ± 3.6 vs 0.08 ± 3.5 ; mean [95% CI] difference -0.94 [$-1.84, -0.05$]; $P = 0.039$) (Fig. 5A and B).

3.1.4. Post hoc efficacy analyses

In subjects with clinically relevant (≥ 14) combined corneal and conjunctival staining at baseline, OM3 was associated with significant improvements in combined staining from baseline at all time points ($P \leq 0.040$), whereas the ROA group only showed a significant improvement from baseline at day 90 ($P = 0.009$). There were no significant between-group differences at any time point, although a directional difference in favor of OM3 was evident at day 30 ($P = 0.089$; Fig. 4B). In subjects with mild/moderate (< 14) combined staining at baseline, statistically significant within-group improvements in combined staining were observed at days 30, 60, and 90 in the OM3 group

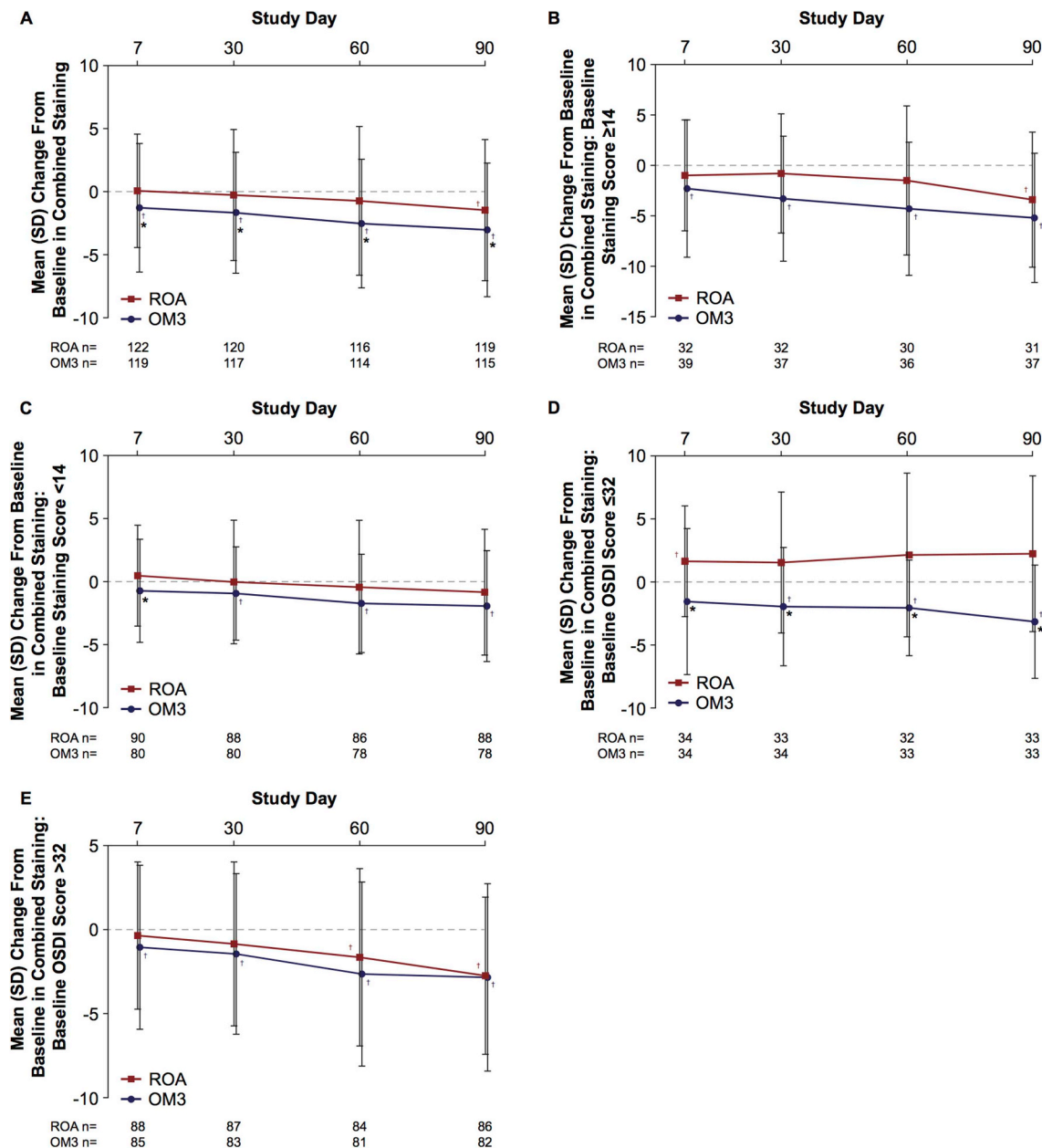


Fig. 4. Mean ($\pm$ SD) change from baseline in combined ocular surface staining: in all subjects (A); in subjects with clinically relevant baseline staining (score ≥ 14) (B); in subjects with mild/moderate baseline staining (score < 14) (C); in subjects with mild/moderate baseline OSDI score (≤ 32) (D) and severe baseline OSDI score (> 32) (E). † $P < 0.05$, within-group differences; * $P < 0.05$, between-group differences. OM3, Refresh Optive MEGA-3 Preservative-Free Lubricant Eye Drops; OSDI, Ocular Surface Disease Index; ROA, Refresh Optive Advanced Preservative-Free Lubricant Eye Drops; SD, standard deviation.

only ($P \leq 0.038$); a significant between-group difference in favor of OM3 was observed at day 7 (mean [95% CI] difference: 1.24 [-2.46, -0.03]; $P = 0.045$), and a directional difference was observed at day 60 ($P = 0.064$) and day 90 ($P = 0.085$; Fig. 4C).

When subjects were stratified by baseline OSDI, within-group changes from baseline in combined staining were significant at days 30, 60 and 90 in the OM3 group ($P \leq 0.018$), and at day 7 only in the ROA group ($P = 0.047$) among subjects in the mild/moderate subgroup (OSDI ≤ 32). Between-group differences were significantly in favor of OM3 at each time point ($P \leq 0.016$) (Fig. 4D); at day 90, the mean $\pm$ SD change from baseline was -3.2 ± 4.5 and -2.8 ± 4.7 in the OM3 and ROA groups, respectively, a mean (95% CI) treatment difference of -5.36 ($-8.02, -2.71$), $P < 0.001$. Within-group changes from baseline in combined staining were significant at each timepoint in the OM3 group ($P \leq 0.039$), and at days 60 and 90 in the

ROA group ($P \leq 0.004$) in the severe (> 32) baseline OSDI subgroup. No significant between-group differences were identified; at day 90, the mean $\pm$ SD change from baseline was -2.9 ± 5.6 and -2.8 ± 4.7 in the OM3 and ROA groups, respectively (Fig. 4E).

Change from baseline in corneal staining in subjects with clinically relevant baseline corneal staining (≥ 7) was significant at each timepoint in the OM3 group only ($P < 0.001$). Statistically significant ($P \leq 0.034$) differences in favor of OM3 were observed at each follow-up visit. In the mild/moderate (< 7) baseline corneal staining cohort, change from baseline was significant at day 90 in the OM3 group ($P < 0.001$); between-group differences were nonsignificant (Fig. 5C and D). Among subjects with mild/moderate baseline OSDI (≤ 32), change from baseline in corneal staining was significant at each timepoint in the OM3 group only ($P \leq 0.043$), and change from baseline in conjunctival staining was significant at days 60 and 90 in the OM3

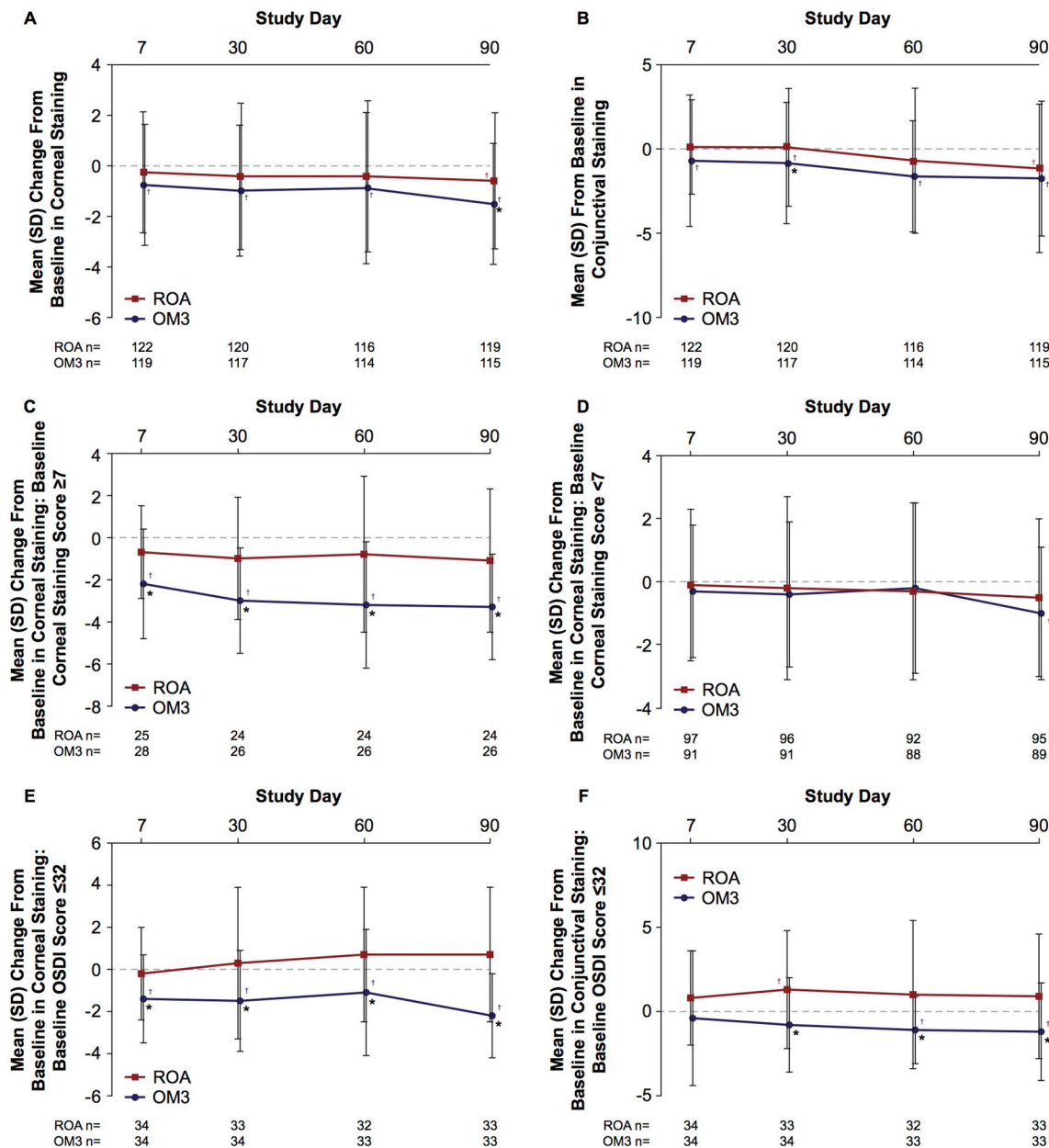


Fig. 5. Mean ($\pm$ SD) change from baseline in corneal and conjunctival staining; corneal staining in all subjects (A); conjunctival staining in all subjects (B); corneal staining in subjects with clinically relevant baseline corneal staining ≥ 7 (C); and mild/moderate baseline corneal staining < 7 (D); corneal staining in subjects with mild/moderate baseline OSDI ≤ 32 (E); conjunctival staining in subjects with mild/moderate baseline OSDI ≤ 32 (F). $\dagger P < 0.05$, within-group differences; $* P < 0.05$, between-group differences. OM3, Refresh Optive MEGA-3 Preservative-Free Lubricant Eye Drops; OSDI, Ocular Surface Disease Index; ROA, Refresh Optive Advanced Preservative-Free Lubricant Eye Drops; SD, standard deviation.

group ($P \leq 0.022$), and day 30 in the ROA group ($P = 0.038$). Significant between-group differences in corneal staining in favor of OM3 were observed at all time points ($P \leq 0.023$) and in conjunctival staining at days 30, 60, and 90 ($P \leq 0.016$) (Fig. 5E and F). However, no significant between-group differences in corneal or conjunctival staining were observed in the severe baseline OSDI group (> 32).

In post hoc analyses of subjects with short TBUT (≤ 5 seconds), change from baseline in combined staining was significant at days 30, 60 and 90 in the OM3 group ($P \leq 0.013$); OM3 ($n = 70$) performed significantly better than ROA ($n = 74$), in reducing combined ocular surface staining at days 60 (mean [95% CI] difference: -2.18 [-4.08 , -0.28]; $P = 0.025$) and 90 (-2.74 [-4.49 , -1.00]; $P = 0.002$) (Fig. 6A). Change from baseline in corneal staining was significant at each timepoint in the OM3 group ($P \leq 0.024$); a significant between-group difference in favor of OM3 was evident at day 90 (mean [95% CI]

difference: -1.06 [-2.00 , -0.12]; $P = 0.027$) (Fig. 6B). OSDI (OM3, $n = 71$ and ROA, $n = 74$) was significantly improved from baseline in both groups at all follow-up visits (Fig. 6C; $P < 0.001$). For TBUT (OM3, $n = 70$ and ROA, $n = 74$), with the exception of a directional improvement at day 60 for the ROA group ($P = 0.06$), both groups demonstrated a significant improvement from baseline at all other follow-up visits ($P < 0.001$; Fig. 6D). However, there was no significant difference between the two treatment groups in the OSDI and TBUT at any time point.

3.1.5. Safety, tolerability, and acceptability

In the safety population, at least one treatment-emergent AE was reported by a similar percentage of subjects in the OM3 (21.7%) and ROA (21.3%) groups. Treatment-related AEs were reported by 6.7% of

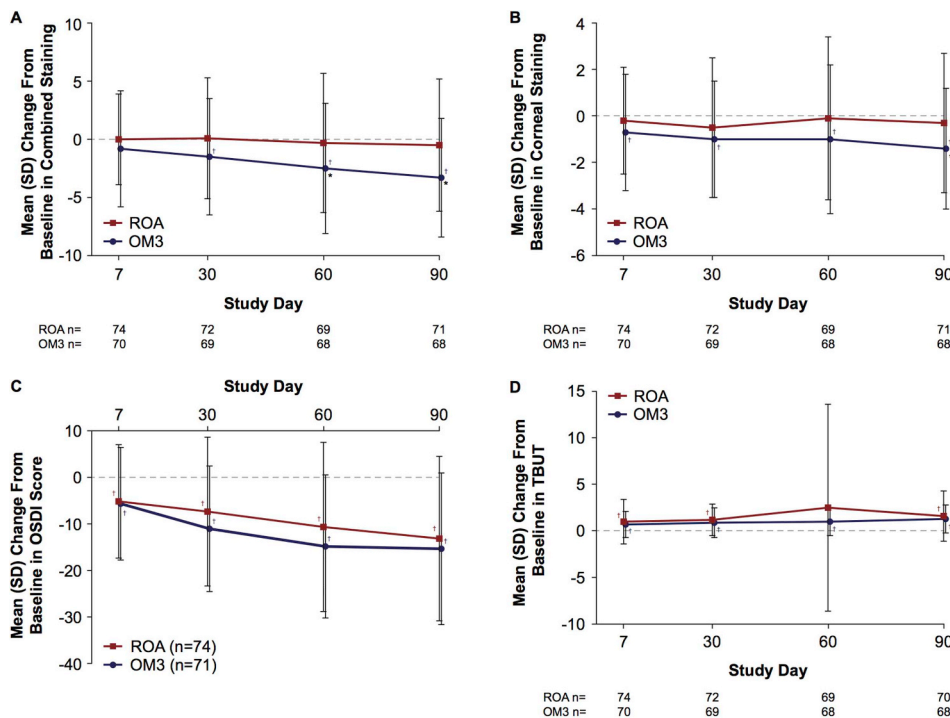


Fig. 6. Mean ($\pm$ SD) change from baseline in combined ocular staining (A); corneal staining (B); OSDI score (C); and TBUT (D) in subjects with a short TBUT (baseline TBUT $\leq$ 5 seconds). $\dagger P < 0.05$, within-group differences; $* P < 0.05$, between-group differences. OM3, Refresh Optive MEGA-3 Preservative-Free Lubricant Eye Drops; OSDI, Ocular Surface Disease Index; ROA, Refresh Optive Advanced Preservative-Free Lubricant Eye Drops; SD, standard deviation; TBUT, tear break-up time.

Table 2
Treatment-related adverse events (safety population).

Preferred term	OM3 (n = 120) n (%)	ROA (n = 122) n (%)
Overall	8 (6.7)	12 (9.8)
Vision blurred	0	5 (4.1)
Conjunctival hyperemia	2 (1.7)	2 (1.6)
Instillation site pain	0	4 (3.3)
Eye irritation	1 (0.8)	2 (1.6)
Instillation site pruritus	2 (1.7)	0
Eyelid margin crusting	1 (0.8)	1 (0.8)
Eye pruritus	0	2 (1.6)
Chalazion	1 (0.8)	0
Foreign body sensation in eyes	1 (0.8)	0
Eye pain	0	1 (0.8)
Instillation site foreign body sensation	0	1 (0.8)

OM3, Refresh Optive MEGA-3 Preservative-Free Lubricant Eye Drops; ROA, Refresh Optive Advanced Preservative-Free Lubricant Eye Drops.

subjects in the OM3 group and 9.8% in the ROA group. The most commonly reported treatment-related AEs ($\geq 1\%$ in any group) were blurred vision (OM3 0% vs ROA 4.1%), conjunctival hyperemia (OM3 1.7% vs ROA 1.6%), instillation site pain (OM3 0% vs ROA 3.3%), eye irritation (OM3 0.8% vs ROA 1.6%), instillation site pruritus (OM3 1.7% vs ROA 0%), and eye pruritus (OM3 0% vs ROA 1.6%) (Table 2). Overall, one subject (0.8%) in each treatment group experienced a serious AE that was considered unrelated to the treatment: diverticulum and colitis ischemia (day 7; OM3) and fall and facial bones fracture (day 54; ROA). Four subjects in each treatment group had a clinically significant biomicroscopy finding (> 1 -grade increase/worsening from baseline), all of which were moderate in severity. The most common clinically significant biomicroscopy finding was conjunctival hyperemia (OM3, n = 2 [1.7%]; ROA, n = 3 [2.5%]).

For the Current Symptom Survey, within-group improvements for each symptom were statistically significant for both treatment groups at days 30, 60, and 90 ($P < 0.05$). A statistically significant between-group difference was observed only for the symptom of burning/stinging at day 60, in favor of the OM3 group ($P = 0.035$). When all

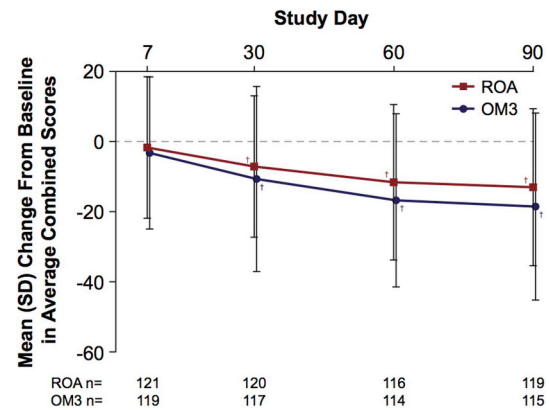


Fig. 7. Mean ($\pm$ SD) change from baseline in the average of the scores for the 5 symptoms on the Current Symptom Survey (post hoc). $\dagger P < 0.05$, within-group differences. OM3, Refresh Optive MEGA-3 Preservative-Free Lubricant Eye Drops; ROA, Refresh Optive Advanced Preservative-Free Lubricant Eye Drops; SD, standard deviation.

symptoms were considered, the average of the scores for the five symptoms showed statistically significant ($P \leq 0.001$) within-group improvements from baseline at days 30, 60, and 90 in both treatment groups (Fig. 7). No statistically significant between-group differences were observed at any follow-up visit, but directional between-group differences in favor of OM3 were observed at day 60 ($P = 0.093$) and day 90 ($P = 0.084$).

Tolerability questionnaire responses in both treatment groups at day 90 indicated improved comfort, soothing, moistening/lubricating, and clear vision, and decreased stickiness, blur, burning/stinging, and discomfort, with no statistically significant difference observed between the two products.

4. Discussion

In this randomized trial, OM3 was found to be noninferior to ROA in reducing dry eye symptom severity after 3 months of treatment. Despite a 7-day run-in period with topical CMC 0.5%, both emulsified artificial

tear formulations were associated with a significant improvement from baseline in OSDI score, ocular surface staining, and TBUT at most follow-up visits. Significant between-group differences, in favor of the OM3 formulation, were observed for the combined corneal and conjunctival staining score at all follow-up time points, corneal staining at day 90, and conjunctival staining at day 30. In post hoc analyses, between-group differences in favor of the OM3 formulation were observed for combined staining at day 7, in subjects with mild/moderate combined staining (< 14) at baseline, and for corneal staining at each visit, in subjects with clinically relevant corneal staining at baseline (≥ 7). These findings suggest that the overall benefit in combined ocular surface staining score might be driven by the difference in improving corneal staining in subjects with clinically relevant staining at baseline.

Additionally, OM3 was observed to perform significantly better than ROA with respect to combined ocular surface staining, corneal staining, and conjunctival staining in individuals with a baseline OSDI score ≤ 32 (non-severe symptoms) and with respect to combined ocular staining and corneal staining in subjects with a short TBUT (defined as < 5 seconds [26,27] at baseline). A short TBUT is often associated with tear film lipid layer deficiency, which causes excessive tear evaporation, and thus is typically termed “evaporative dry eye” [26,27]. The observed clinical benefits were reflected in subject-reported improvements with both treatments, in terms of dry eye symptoms and formulation tolerability, although the only subject-reported treatment difference that was statistically significantly in favor of OM3 was burning/stinging reported on the Current Symptom Survey at day 60. This finding is consistent with the known poor correlation between signs and symptoms in dry eye disease [28].

Both dry eye formulations were well tolerated, although treatment-related AEs of blurred vision and instillation site pain occurred more often in the ROA group compared with the OM3 group. The absence of blurred vision reported with OM3, in contrast to ROA, may be reflective of the nano-emulsion structure of the new formulation, consisting of ultrafine dispersions with viscoelastic and translucent properties that promote the comfort and clarity of an aqueous eye drop while minimizing effects on vision. Between-group differences in ocular surface staining in favor of OM3 may also be attributable to the additional presence of flaxseed oil and/or trehalose, both of which have demonstrated benefits in treating dry eye symptoms.

In a mouse model of dry eye disease, the addition of flaxseed oil (short-chain omega-3 fatty acids) to hyaluronic acid eye drops reduced corneal epithelial barrier disruption and decreased levels of ocular surface proinflammatory markers compared with hyaluronic acid therapy alone [29]. In a separate experimental model of dry eye [30], topical short-chain omega-3 fatty acid treatment induced a reduction in both the number of corneal dendritic cells and the expression of conjunctival proinflammatory mediators. Similar benefits, relating to a reduction in tear proinflammatory cytokines with a topical long-chain omega-3 fatty acid preparation, have recently been documented in individuals with contact lens discomfort [20]. This raises the possibility of topical omega-3 fatty acids acting through a local, ocular pathway to impart anti-inflammatory effects.

Supporting a role for omega-3 fatty acids in tear film dysfunction, observational studies have reported associations between high dietary omega-3 fatty acid consumption and a reduced risk of dry eye disease [31]. Dietary omega-3 fatty acid intake can influence the polar lipid pattern of meibomian gland secretions in people with Sjögren's syndrome [32]. In addition, the ratio of omega-6 to omega-3 fatty acids in the tear film is altered in individuals with dry eye disease relative to those with healthy tear films [33]. A recent meta-analysis and systematic review reported a potential benefit with oral omega-3 fatty acid supplementation in individuals with dry eye disease, in terms of improvements in TBUT and Schirmer test score [34]. However, the recent Dry Eye Assessment and Management multicenter clinical trial, which assessed the effects of high-dose fish oil supplementation (eicosapentaenoic acid 2000 mg/day + docosahexaenoic acid 1000 mg/day),

did not identify a clinical benefit in individuals with moderate to severe symptoms of dry eye disease, as compared with the placebo supplement (olive oil: 5000 mg/day) [19]. Although the reason for this negative finding is not certain, it may be attributable to one or more factors, including the characteristics of the study population, choice of comparator, and level of control over concurrent medications and procedures for dry eye during the study.

It is likely that apparent differences in therapeutic efficacy may be related to the dose and form of omega-3 fatty acid supplementation (and the selected placebo intervention), and definitions relating to the enrolled study populations (eg, disease severity, use of concomitant therapies). The potential benefit of long-chain omega-3 fatty acids for the treatment of dry eye symptoms has been shown to be influenced by their source and type, with evidence that oral supplements in predominantly phospholipid form (eg, krill oil supplements) may be more effective for modulating ocular surface inflammation than supplements in triacylglyceride or ethyl ester form (eg, fish oil supplements) [16]. As recently reviewed, most clinical studies that have investigated omega-3 fatty acid interventions for the treatment of dry eye disease have evaluated long-chain (ie, eicosapentaenoic acid and docosahexaenoic acid) rather than short-chain (alpha-linolenic acid) forms of supplementation [10]. As short-chain omega-3 fatty acids require an extra metabolic step in vivo to yield biologically active metabolites, long-chain forms are generally considered preferable for oral consumption; however, the relative therapeutic efficacy of these subtypes in topical form remains unknown. Hypothesized mechanisms of action, considered to underlie the potential clinical benefits of omega-3 fatty acids, include the promotion of stem cell proliferation and differentiation (to improve ocular surface epithelial integrity), as well as anti-inflammatory and neuroprotective properties [35]. Together, these factors may explain the observed protective effect of omega-3 fatty acids on corneal sensory nerves and the correlation with tear osmolarity normalization [17].

Similarly, trehalose is associated with significant improvements in ocular surface staining and TBUT [36] and was shown to outperform artificial tears containing hyaluronan or hydroxyethylcellulose in a clinical study of moderate-to-severe dry eye disease [37]. Trehalose is a disaccharide and was included in the OM3 formulation as an antioxidant to stabilize the flaxseed oil [38]. In addition to antioxidation, it also serves as an osmoprotectant on the ocular surface, likely through a different mechanism of action than that of small-molecule osmoprotectants like L-carnitine and erythritol. Small-molecule osmoprotectants can enter cells to balance osmotic stress, whereas the trehalose molecule, due to its large size, acts extracellularly, likely at the level of the cell membrane [39]. There is emerging evidence that both small-molecule osmoprotectants such as L-carnitine and erythritol, and large molecules such as trehalose, elicit direct anti-inflammatory/antioxidative effects following hyperosmotic stress [40–43] and have a direct benefit on dry eye symptoms [11].

The limitations of this study include the inability to distinguish whether the observed benefit of OM3 is attributable to flaxseed oil and/or trehalose. To date, no dry eye studies have investigated the potential benefits of topical alpha-linolenic acid omega-3 fatty acids, sourced from flaxseed oil, and different sources of omega-3 have been shown to influence efficacy in dry eye disease.

The study population was typical of dry eye patients who use artificial tears, with the exclusion of individuals with very severe dry eye disease, based on the Schirmer score at screening and staining at screening and/or baseline (after a 7-day run-in with CMC 0.5%). The majority of subjects had evaporative elements in their dry eye etiology, based on a TBUT of ≤ 5 seconds. The chosen 90-day intervention period was based on prior clinical experience and similarity to existing lubricant artificial tears, and dosing was consistent with an “as-needed” label, but with a minimum use of two times per day, to acquire sufficient safety information.

In summary, these data support the application of OM3, a novel

preservative-free, nano-emulsion tear formulation with trehalose and flaxseed oil, in people with dry eye disease, particularly those with a short TBUT suggestive of evaporative dry eye. Most notably, the presence of trehalose and flaxseed oil appears to confer additional protection to the ocular surface as evidenced by the magnitude of the reduction in staining. Future studies could investigate the possible dose-response interaction with the flaxseed oil and trehalose components, to identify their relative contribution to overall therapeutic efficacy. The finding that OM3 performed better in individuals with severe (corneal staining) signs, yet non-severe (OSDI) symptoms, also warrants further investigation.

Funding/disclosures

This study was funded by Allergan plc, Irvine, CA. L. Downie has received financial support from Alcon, Allergan, CooperVision and Azura Ophthalmics. M. Hom has received financial support from Allergan, Bausch & Lomb, and Takeda. G. Berdy has received financial support from Alcon, Allergan, Bausch & Lomb, Parion, Shire, and Sun Pharma. S. El-Harazi has received financial support from Allergan. A. Verachtert has received financial support from Allergan. J. Tan has received financial support from Alcon, Allergan, Azura Ophthalmics, CooperVision, Menicon, and Ophtecs. H. Liu and C. Carlisle-Wilcox are employees of Allergan plc, Irvine, CA. P. Simmons and J. Vehige were employees of Allergan plc, Irvine, CA, at the time the study was conducted.

Medical writing and editorial assistance were provided to the authors by Stuart Murray and Scarlett Geunes-Boyer of Evidence Scientific Solutions, Inc, Philadelphia, Pennsylvania, and funded by Allergan plc, Dublin, Ireland. All authors met the ICMJE authorship criteria. No honoraria or payments were made for authorship.

Acknowledgments

The authors would like to thank the study participants and the following study sites: Canyon City Eyecare, California, US; Havana Research Institute, California, US; Eugene Eye Institute, California, US; The Private Practice of Eric M. White, California, US; Moyes Eye Center, Missouri, US; Ophthalmology Associates, Missouri, US; Rochester Ophthalmological Group, New York, US; Charlotte Eye Ear Nose, North Carolina, US; Scott and Christie and Associate, Pennsylvania, US; Texan Eye, Texas, US; Department of Optometry and Vision Sciences, The University of Melbourne, Victoria, Australia; Private Practice of M. T. Coroneo, New South Wales, Australia; and School of Optometry and Vision Science, The University of New South Wales, Sydney, Australia). The following institutional review boards and ethics committees granted protocol approval: Schulman IRB, Cincinnati, OH, USA; The University of Melbourne Office for Research Ethics and Integrity, Melbourne, Australia; Bellberry Human Research Ethics Committee, Eastwood SA 5063, Australia; University of New South Wales Human Research Ethics Committee, Sydney, Australia.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtos.2019.11.004>.

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