# Study protocol

Efficacy and Safety of a Multiple-Action Tear Substitute (TriMix) in Dry Eye Disease: A Randomized, Double-Blinded, HA-controlled Study

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### *1. Study design*

This was a randomized, double-masked, HA-controlled clinical trial conducted in the ophthalmology departments of Tedesco Eye Center (Girifalco, CZ, Italy) and Russo multi-specialty medical practice (Corso Garibaldi, CZ, Italy) between July, 2023 and May, 2024. The study protocol fulfilled all the requirements of the Declaration of Helsinki. Before initiating the study, informed and written consent was obtained from each patient.

### *2. Participants*

Eligible patients were adults ≥ 18 years old with a self-reported history DED while working with computer screens ≥ 6 hours per day. Patients had to meet the following inclusion criteria in ≥ 1 eye at screening and randomization to be enrolled in the study: (1) ocular surface disease index (OSDI) > 13 points; (2) non-invasive tear film break-up time (NIBUT) < 10 s; (3) Schirmer test (ST) without anesthesia ≥ 5 mm and (4) MGD grade ≤ 1. For MGD, the Sirius device (CSO, Florence, Italy) was used, which determines MGD grade based on loss area of meibomian glands (LAMG). MGD grade was scored from 0 to 4 (MGD grade 1 = LAMG < 25%; MGD grade 2 = LAMG ≥ 25% and < 50%; MGD grade 3 = LAMG ≥ 50% and < 75%; MGD grade 4 = LAMG ≥ 75%).

Patients were excluded from the study if they met any of the following criteria: (1) abnormal lid anatomy, including active blepharitis, and active lid margin; (2) all corneal disorders that affect diagnostic test, such as active corneal infection and corneal dystrophies; (3) active ocular allergies; (4) vectored thermal pulsation (VTP) intense pulse light (IPL), quantum molecular resonance (QMR), or other procedure to treat DED within the previous 6 months; (5) intraocular surgery or laser ocular surgery within the previous 6 months; (6) use of topical antibiotics and anti-inflammatory treatments, including steroids and non-steroidal anti-inflammatory drugs; (7) systemic autoimmune diseases; (8) contact lens wearers; (9) pregnant or lactating women; (10) patients who did not understand or comprehend the informed consent. Prior use of tear substitutes was allowed, and there was a one-month washout period after enrollment.

### *3. Randomization, intervention and masking*

Eligible participants were randomized in a 1.5:1 ratio to receive either TriMix tear substitute (Off Health S.p.a., Firenze, Italy) or 0.15% HA tear substitute (Thea Pharma S.p.a., Milan, Italy). Randomization tables were generated by an unmasked statistician not otherwise involved in the trial using the software "randomiser" (Appier inc., Taiwan, China). Patients were instructed to instill 1 drop TriMix tear substitute or 0.15% HA tear substitute into each eye 3 times per day for 6 months. HA-based tear substitutes can reduce tear film hyperosmolarity and are effective in treating DED. Therefore, 0.15% HA tear substitute was selected as a suitable comparator. Both tear substitutes are transparent, with no special smell and the bottles were identical in appearance such that patients and investigators were masked to treatment assignment.

### **4.** *Clinical outcomes*

The study design is shown in Figure 1. Patients were assessed at screening, baseline (day 1), and 2 follow-up visits: 3 months (12 ± 1.5 weeks) and 6 months (24 weeks ± 1). All clinical outcomes were conducted in the order suggested by Ballesteros et al. to maintain the integrity of the tear film. Furthermore, these measurements were taken under consistent environmental conditions in the same room by a trained optometrist.

### *4.1. Dry eye symptoms*

The OSDI questionnaire were employed to assess the severity of DED symptoms, with scores ranging from 0 (indicating no ocular surface disease) to 100 (indicating severe ocular surface disease) points [1,2]. This questionnaire was provided during consultations at each follow-up visit.

### *4.2. Tear film stability*

Tear film stability was automatically assessed using NIBUT by projecting Placido rings from the Sirius device (CSO, Florence, Italy) onto the corneal surface. The time interval between the last blink and the initial distortion of the ring pattern was defined as first NIBUT. This variable was always measured at least 12 hours after administration of the study medication and the average of 3 consecutive measurements was calculated for statistical analysis.

### *4.3. Tear film volume*

Tear film volume was evaluated with unanesthetized ST. During the test, the patient is instructed to look upward while the test strip is carefully positioned between the palpebral conjunctiva of the lower eyelid and the bulbar conjunctiva. Subsequently, the patient is asked to keep their eyes gently closed for five minutes. After this period, the test strip is removed, and the Schirmer test score is determined by measuring the length of the moistened area on the strip.

### *5. Statistical analysis*

Statistical analyses were performed with SPSS statistics software, version 25.0 (IBM Corporation, NY, USA). The sample size was estimated using the GRANMO calculator, version 7.12 (Municipal Institute of Medical Research, Barcelona, Spain). It was calculated based on a statistically significant paired difference of 1.24 ± 2.56 s at 2 months after the TriMix tear substitute treatment onset. This assumed difference was based on the findings of a pilot study with 25 eyes [3]. With these assumptions, a sample size of 40 eyes per group would yield a power > 80% and a statistically significant paired difference of 95% confidence. A 20% discontinuation rate was considered when calculating sample size. Continuous variables were displayed as the mean ± standard deviation (SD), while ordinal categorical variables were expressed as frequencies (n) and percentages (%). When both eyes were eligible, the eye with a lower baseline NIBUT was selected for the analyses. If the baseline NIBUT was the same in both eyes, the right eye was selected. Since the Kolmogorov-Smirnov test showed that the clinical endpoints did not meet the normality criteria, nonparametric tests were performed. The Friedman test (non-parametric) was performed to compare intra-group clinical outcomes. Within each group, the increment (Δ) was calculated. It was defined as the change from the last visit (LV) to baseline (B): D = LV – B. Inter-group clinical outcomes were analyzed with the Mann–Whitney U test. Between each group, the differences were calculated as $\Delta_{\text{TriMix group}} - \Delta_{\text{HA group}}$. Categorical variables were compared using the $\chi^2$ test. A *P* value of less than 0.05 is considered to be statistically significant.

***References***

[1] Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. Arch Ophthalmol 2000;118:615–21. https://doi.org/10.1001/ARCHOPHT.118.5.615.

[2] Dougherty BE, Nichols JJ, Nichols KK. Rasch analysis of the Ocular Surface Disease Index (OSDI). Invest Ophthalmol Vis Sci 2011;52:8630–5. https://doi.org/10.1167/IOVS.11-8027.

[3] Vigo L, Senni C, Pellegrini M, Vagge A, Ferro Desideri L, Carones F, et al. Effects of a New Formulation of Multiple-Action Tear Substitute on Objective Ocular Surface Parameters and Ocular Discomfort Symptoms in Patients with Dry Eye Disease. Ophthalmol Ther 2022;11:1441–7. https://doi.org/10.1007/S40123-022-00518-7.