Title: Efficacy of Low-Level Light Therapy in Combination with Topical Non-Steroidal Immunosuppressants for the Treatment of Dry Eye Disease: A Multicenter, Randomized, Single-Masked, Active-Controlled Trial.

NCT number: ID not yet assigned.

Date: 10/13/2025.

Study protocol

This was a randomized, single-masked, active-controlled trial conducted at Centro Oculistico Borroni (Varese, Gallarate, Italy) and Biomeeting Day Surgery center (Calabria, Reggio Calabria, Italy) between May, 2024 and June 2025. The study protocol was approved by the institutional review board of the Riga Stradins University (nr.29/20092016**)** and adhered to the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrollment. The study was registered at ClinicalTrials.gov (identifer, XXX) and follows the Consolidated Standards of Reporting Trials (CONSORT) guidelines [26].

Participants

Participants eligible for inclusion were adults ≥ 25 years old with a self-reported history DED. To be enrolled in the study, participants were required to meet the following inclusion criteria in at least one eye: (1) Ocular Surface Disease Index (OSDI) score > 13; (2) non-invasive tear film break-up time (NIBUT) < 10 seconds; and (3) tear meniscus height < 0.25 mm. Participants were excluded if they met any of the following conditions: (1) structural abnormalities of the eyelids; (2) active blepharitis; (3) corneal disorders that could interfere with study assessments, such as active corneal infections or corneal dystrophies; (4) active ocular allergy; (5) history of procedures for DED treatment within the previous 12 months, such as eyelid exfoliation, thermal eyelid therapies, light therapies, or quantum molecular resonance (QMR); (6) history of intraocular or laser ocular surgery within the past 5 years; (7) current use of topical antibiotics or anti-inflammatory agents; (8) diagnosis of systemic autoimmune disease; (9) contact lens wear; (10) pregnancy or lactation; and (11) inability to understand or provide informed consent. Prior use of tear substitutes was allowed, and there was a 4-week washout period after enrollment.

Randomization, intervention, and masking

Eligible participants were randomized in a 1:1:1:1 ratio to receive either topical CsA 0.1% (CsA group), topical Tacrolimus 0.1% (Tacrolimus group), LLLT combined with topical CsA 0.1% (LLLT–CsA group), or LLLT combined with topical Tacrolimus 0.1% (LLLT–Tacrolimus group). A random allocation sequence was generated using Randomizer.app (PWA Labs Inc., CA, USA) by an ophthalmologist who was not otherwise involved in the study. The same individual was responsible for performing the LLLT procedure and providing CsA 0.1% or Tacrolimus 0.1% according to group allocation. Treatment allocation was concealed from the ophthalmologists and optometrists involved in the study until the conclusion of data analysis.

- Photobiomodulation: LLLT was administered using the eye-light® device (Espansione Group, Bologna, Italy), which employs Light Modulation™ technology. The treatment was delivered with a wavelength of 625 nm and a radiance of 35 mW/cm², resulting in a total energy dose of approximately 32 J/cm² applied to the facial and eyelid regions with eyes

closed. Three 15-minute sessions were performed at weekly intervals starting at the baseline visit. The same protocol was repeated at the 6-month follow-up, following evaluation of the outcome measures.

- Topical CsA and Tacrolimus preparation: CsA 0.1% and Tacrolimus 0.1% were prepared as sterile aqueous microemulsions. The formulation excluded ethanol and preservatives, using the the most functional and minimal number of excipients to minimize adverse events: ethoxylated hydrogenated castor oil, polysorbate 80, sodium chloride, sodium hyaluronate, phosphate buffer (pH: 6.8 - 7.2), and water for injections. A concentrated 10 mg/mL emulsion was first prepared in a biological safety cabinet, then mixed under sterile cleanroom conditions with a buffered base solution containing the other excipients. Final sterilization was performed using 0.22 μm filtration under a Grade A hood, followed by packaging in sterile dropper bottles. CsA 0.1% and Tacrolimus 0.1% were clear and odorless, thereby minimizing sensory differences for the participants. Moreover, the bottles were identical in both appearance and labeling, which helped ensure that patients remained masked to their treatment allocation. Patients were instructed to apply the medications every 12 hours.

Assessment of the outcome measures

The study design is shown in Fig. 1. Patients were assessed at screening, baseline (day 1), and 2 follow-up visits: 6 months (24 ± 2.5 weeks) and 12 months (48 ± 2.2 weeks). The primary efficacy outcome measure was the change from baseline at months 6 and 12 in OSDI score. The OSDI questionnaire was performed during each follow-up visit as part of the clinical consultation. The secondary efficacy outcome measures were the change from baseline at months 6 and 12 in tear film stability and tear volume, assessed using the Sirius device (CSO, Florence, Italy). Tear film stability was automatically assessed through non-invasive tear film break-up time (NIBUT) by projecting Placido rings from the Sirius device onto the corneal surface. The time interval between the last blink and the initial distortion of the ring pattern was defined as first NIBUT. For statistical analysis the average of 3 consecutive measurements was calculated for each participant. Tear volume was evaluated with TMH using the tear film scanning function of the Sirius device. Measurements were performed below the corneal vertex at the 6 o'clock position using the device's built-in software calliper tool. The Sirius device was located in the same examination room throughout the study period, with controlled temperature and humidity conditions. Outcome measures were conducted under scotopic conditions, at the same time of day to minimize diurnal variability of TMH. All measurements were performed by a well-trained optometrist.