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.

Statistical analysis

Statistical analyses were conducted with SPSS statistics software, version 25.0 (IBM Corp., Armonk, NY, USA). The sample size was estimated using the GRANMO calculator, version 8.0 (Registre Gironí del Cor, Barcelona, Spain). It was calculated based on assumed mean difference in OSDI scores between groups at 6 months after the intervention onset, with a mean value of -8.05 ± 2.78 points (Range: -0.75 to -12.74). This assumed difference were based on the findings of a pilot study with 20 eyes of 10 patients in each group. With these assumptions, a sample size of 24 eyes per group would yield a power > 80% and a statistically significant paired difference of 95% confidence. A 5% lost to follow-up was considered when calculating sample size.

The Kolmogorov-Smirnov test confirmed that the outcome measures met the normality criteria, allowing for the application of parametric tests. A repeated measures ANOVA with Bonferroni-adjusted post hoc tests was conducted to assess intra-group changes. For each group, the change (Δ) was calculated for the intervals between baseline and month 6 ($M6$), baseline and month 12 ($M12$), and month 6 to month 12 ($M6 \rightarrow M12$), using the following formulas:

$$\Delta_{M6} = M6 - Baseline \qquad \Delta_{M12} = M12 - Baseline \qquad \Delta_{M6 \rightarrow M12} = M12 - M6$$

A one-way ANOVA with Bonferroni-adjusted post hoc tests was also performed to compare inter-group differences. The difference between the changes (D_{Δ}) observed within each group over the mentioned intervals was computed for each pair of groups, using the following formulas:

$$D_{\Delta M6} = \Delta_{M6}^{(x)} - \Delta_{M6}^{(y)} \qquad D_{\Delta M12} = \Delta_{M12}^{(x)} - \Delta_{M12}^{(y)} \qquad D_{\Delta M6 \rightarrow M12} = \Delta_{M6 \rightarrow M12}^{(x)} - \Delta_{M6 \rightarrow M12}^{(y)}$$

$$For\ all\ x \neq y \rightarrow x, y \in \{CsA, Tacrolimus, LLLT{-}CsA, and\ LLLT{-}Tacrolimus\ groups\}$$

Categorical variables were compared using the Chi-squared test ($\chi 2$). Statistical significance and clinical relevance were evaluated. Statistical significance was defined as a *P*-value less than 0.05. Clinical relevance was estimated using partial eta squared (η_p^2) for ANOVAs, Cohen's d_z for within-group comparisons, and Cohen's d_s for between-group comparisons in Bonferroni-adjusted post hoc tests. According to Cohen's benchmarks, effect sizes were classified as small ($\eta_p^2 < 0.06$; $d_z = d_s < 0.5$), moderate ($\eta_p^2 \geq 0.06$ and < 0.14; $d_z = d_s \geq 0.50$ and < 0.8), and large ($\eta_p^2 \geq 0.14$; $d_z = d_s \geq 0.80$).