Supplementary Methods for "Topical Timolol 0.5% Gel-Forming Solution for Erythema in Rosacea: A Quantitative, Split-Face, Randomized, and Rater-Masked Pilot Clinical Trial"

Study Participants

This study was approved by the Johns Hopkins University Institutional Review Board. Participants provided written informed consent prior to participation. 12 adult participants diagnosed by a dermatologist with rosacea involving frequent flushing and persistent facial erythema were enrolled in Baltimore, Maryland. Additional inclusion criteria included age 18-65 years and willingness to not take any other medication during the study. Exclusion criteria included history of taking any investigational drug within 30 days of study entry, use of systemic or topical medications for rosacea including antibiotics within three weeks, current or planned pregnancy, lactation, severe depression, and inability to provide informed consent. Participants with history of hypersensitivity to beta-blockers, hypotension, bradycardia, congestive heart failure, myocardial infarction, arrythmia, asthma, and bronchospasm were excluded.

Quantification of Erythema with Tristimulus Colorimetry

At each visit, facial erythema was measured with the Chroma Meter CR-400 colorimeter (Konica Minolta Sensing Americas, Inc., NJ, USA), which includes a D65 illuminant and measuring head with 8 mm measurement area. The device was set to the Commission Internationale de l'eclairage (CIE) L* a * b* mode. Participants were allowed to equilibrate by resting for ≥ 15 minutes, and facial skin was cleansed with alcohol wipe prior to measurement. For each measurement, the colorimeter was calibrated with a white reference panel and pressed perpendicularly against the malar cheek (standardized to the intersection of the mid-pupillary

line and line drawn laterally from the ipsilateral nasal ala) with moderate pressure, with care taken to minimize skin blanching. Averaged triplicate readings of L*, a*, and b* values were obtained for each side of the face; L* indicates lightness ranging from 0 (black) to 100 (white), a* indicates red-green chromaticity ranging from -60 (green) to +60 (red), and b* indicates blue-yellow chromaticity ranging from -60 (blue) to +60 (yellow). Colorimeter-measured a* values were used to represent erythema, with more positive values suggestive of increased erythema.

Quantification of Erythema with Computer-Aided Image Analysis

At each visit, cross-polarized photographs of both sides of the face were taken with a Canon EOS 5D Mark II camera (Canon, Japan) and VISIA-CR Facial Imaging Booth (Canfield Scientific, NJ, USA). The ImageJ software version 1.53a was used to convert each photograph to separate CIE L*, a*, and b* 8-bit grayscale images. Pixel intensities of L*, a*, and b* images ranged from 0 to 255 and cover the black-white, green-red, and blue-yellow gradients, respectively. For each a* image, erythema was calculated by manually outlining the cheek (encompassing the infraorbital, zygomatic, and mandibular regions) and measuring mean pixel intensity, with more positive values indicative of increased erythema.

Sample Size Calculation

Sample size calculation was performed to detect a difference in colorimeter-measured a* of 3.6 between affected and unaffected areas using the paired-sample t-test, assuming a standard deviation of 3 based on previously published measurements. Type I error rate and power were set at 0.05 and 0.8, respectively, yielding a required sample size of eight participants in each treatment arm.

Analysis of Change in Erythema with Mixed-Effects Models

Mixed-effects models were used to characterize change in erythema over time, with participants included as random effect. Timepoint of visit was treated as a categorical variable, since change in erythema over time was expected to be nonlinear due to initiation of treatment on the delayed treatment side at week 8 and discontinuation of treatment on both sides at week 16. All analyses were performed with R version 4.0.0. Mixed-effects models were created using the lme4 package. Comparisons of erythema at each timepoint to baseline were performed with t-tests using Satterthwaite's method with Benjamini-Hochberg correction for multiple comparisons. Visualizations were created with the ggplot2 package. For all analyses, two-sided $P<.05$ was accepted as statistically significant.

Imputation of Missing Data with Maximum Likelihood Method

12 participants were initially enrolled, but four did not return after the baseline visit due to lack of improvement (participant 01), scheduling difficulty (participant 10), and loss to contact (participants 11, 12). The remaining eight participants who completed ≥ 2 visits were included in the final data set (**Table S1**). Participant 07 withdrew after the second visit due to scheduling difficulty. Only the first two visits of participant 06 were included in downstream analyses since the third visit occurred six weeks later than scheduled. Missing data of participants 06 and 07 were treated as missing at random in mixed-effects models and estimated with the maximum likelihood method based on outcomes of participants with similar baseline characteristics.