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Longitudinal Ocular Surface Microstructural Findings After Switching to Preservative-Free Dorzolamide/Timolol in POAG: An Exploratory IVCM Pilot Case Series

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Abstract

Background and Objectives: To describe longitudinal ocular-surface microstructural findings on in vivo confocal microscopy (IVCM) after switching from benzalkonium chloride-preserved dorzolamide 2%/timolol 0.5% (BAK-DT) to preservative-free dorzolamide 2%/timolol 0.5% (PF-DT) in four patients with primary open-angle glaucoma (POAG) and ocular surface disease (OSD). Materials and Methods: This prospectively planned, exploratory pilot case series included four participants (eight eyes) from a previously published 30-participant parent study. IVCM was performed at baseline and months 3, 6, 9, and 12. Because only four independent participants contributed both eyes, analyses were descriptive, and no inferential tests were performed. Results: All four participants completed follow-up. From baseline to month 12, median superficial corneal epithelial cell density increased by 2010.00 cells/mm^2 and median conjunctival epithelial cell density by 3261.5 cells/mm^2; median stromal reflectivity decreased by 0.07 arbitrary units and median dendritic cell density by 75.49 cells/mm^2. Both eyes of each participant changed in the same direction for these four outcomes. Other IVCM measures showed no uniform temporal pattern. Conclusions: The temporal IVCM changes observed after the therapeutic switch were compatible with lower ocular-surface inflammatory activity. These preliminary findings should be interpreted cautiously and confirmed in larger controlled studies.

Keywords: in vivo confocal microscopy; glaucoma; dorzolamide; timolol; preservative-free; ocular surface disease; pilot case series

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1. Introduction

Primary open-angle glaucoma (POAG) is a leading cause of irreversible blindness and frequently requires lifelong topical intraocular pressure-lowering therapy [1–5]. Long-

term exposure to topical medication can compromise the ocular surface and may reduce treatment tolerability and adherence [5].

Benzalkonium chloride (BAK), the preservative used in many multidose glaucoma formulations, can disrupt epithelial tight junctions, promote apoptosis, and amplify inflammatory signaling [6–16]. These effects are relevant in patients with glaucoma because cumulative exposure may coexist with ocular surface disease (OSD) and with toxicity from the active compounds themselves [12,13].

Ocular-surface assessment usually combines symptoms, slit-lamp examination, tear-film testing, and vital dyes such as fluorescein, rose bengal, or lissamine green [17–20]. These measures are clinically useful but may not fully characterize cellular or nerve-level alterations.

In vivo confocal microscopy (IVCM) provides minimally invasive, high-resolution imaging of corneal and conjunctival microstructure, including epithelial cells, dendritic cells, stromal reflectivity, endothelium, and the sub-basal nerve plexus [21–27]. It is therefore useful as a research tool for describing microstructural findings that may not be apparent on routine examination.

Evidence supporting preservative-free glaucoma therapy has continued to develop. Recent randomized phase III data showed that preservative-free latanoprost emulsion maintained intraocular pressure-lowering efficacy while improving selected ocular-surface outcomes compared with preserved latanoprost [28]. Nevertheless, formulation-specific and longitudinal IVCM data remain limited.

We previously reported the clinical outcomes of a 30-participant prospective study in which patients were switched from BAK-preserved dorzolamide 2%/timolol 0.5% (BAK-DT) to preservative-free dorzolamide 2%/timolol 0.5% (PF-DT), with clinical follow-up through week 24 [29]. Those clinical outcomes are not reanalyzed here. The objective of the present, prospectively planned exploratory pilot case series was to describe previously unpublished longitudinal IVCM findings in four participants from the parent study at baseline and at months 3, 6, 9, and 12. Given the very small number of independent participants, the study was framed as descriptive and hypothesis-generating rather than confirmatory.

2. Materials and Methods

This report describes a prospectively planned, exploratory IVCM pilot case series nested within a phase IV, multicenter, open-label, single-arm prospective study conducted in Argentina between March 2023 and October 2024 (protocol code ANTIGLAULC-001) [29]. This study was conducted in Argentina and did not involve investigational medicinal products, unapproved therapeutic indications, or experimental devices. The investigational treatment is commercially available in Argentina, has been approved for clinical use, and was administered in accordance with its authorized indications as part of routine clinical practice. Under the current Argentine regulatory framework, registration in an international clinical trial registry is not required for studies conducted under these conditions. The parent study and the IVCM component were conducted in accordance with the Declaration of Helsinki and applicable local regulations (Resolution 1480/11 of the Ministry of Health of Argentina). The protocol was approved before enrollment by the research ethics committee of the Swiss Argentine Clinic and Maternity and Olivos Clinic (protocol code ANTIGLAULC-001; approval dated 1 March 2023). All participants provided written informed consent.

Subjects had to meet the following inclusion criteria to be eligible for participation in the clinical trial: (1) male or female ≥18 years of age; (2) who provided written informed consent; (3) with primary open-angle glaucoma or bilateral ocular hypertension; (4) who had been under treatment with BAK-preserved Dorzolamide 2–Timolol 0.5% solution for

at least the previous 6 months; (5) with corneal thickness measured by pachymetry within the range of 520 to 580 μm; (6) with ocular surface disease index (OSDI) score greater than 13 points; (7) with intraocular pressure <20 mmHg in both eyes and (8) with at least one of the following clinical signs of dry eye disease: tear break-up time (BUT) <10 s, Schirmer test <5 mm/5 min, or positive corneal staining with more than 5 points of corneal erosion.

Participants were excluded from the study if they met any of the following criteria: (1) history of respiratory diseases; (2) history of cardiac diseases; (3) history of severe renal insufficiency (CrCl < 30 mL/min); (4) any ocular pathology that according to the investigator's judgment could compromise the administration of the study medication or the safe participation in the study; (5) history of progressive retinal or optic nerve disease other than glaucoma; (6) active ocular inflammatory or infectious conditions; (7) progressive cataracts; (8) comorbidities of the ocular surface other than OSD; (9) eyelid disorders; (10) oral use of carbonic anhydrase inhibitors or beta-blockers; (11) oral use of medications that interact with Dorzolamide or Timolol; (12) use of any other topical ocular medication, in addition to the anti-glaucoma medication, especially those for dry eye treatment such as lubricants, corticosteroids, and immunomodulators; (13) regular use of contact lenses; (14) active autoimmune diseases; (15) history of laser keratorefractive procedures, corneal surgery, or corneal surface surgery, within 6 months prior to the baseline visit; (16) history of any intraocular or extraocular laser surgery or surgery on either eye within the 6 months prior to the baseline visit; (17) severe loss of central visual field in either eye (defined as a sensitivity of $\leq$10 dB in at least two of the four closest test points to the fixation point); (18) known hypersensitivity to any component of the study medications; (19) pregnant or lactating women.

At baseline (day 0), participants were switched from a BAK-DT solution to PF-DT (Glaucotensil® TD LC; POEN S.A.U., Buenos Aires, Argentina). The dosing schedule was unchanged (one drop twice daily). The PF formulation is supplied in a multidose ophthalmic squeeze dispenser designed to maintain sterility without preservatives (Aptar Pharma, Crystal Lake, IL, USA).

The following prespecified exploratory IVCM outcomes were assessed: Superficial corneal epithelial cell density; Basal corneal epithelial cell density; Superficial conjunctival epithelial cell density; Stromal reflectivity; Endothelial cell density; Dendritic cell density; Sub-basal nerve length and tortuosity. IVCM assessments were performed at baseline and at months 3, 6, 9, and 12.

Participants were examined with a digital laser-scanning confocal microscope (HRT II Rostock Cornea Module; Heidelberg Engineering GmbH, Heidelberg, Germany) using a 670 nm diode laser. The device produces 400 × 400 μm frames at ×400 magnification, with a manufacturer-specified lateral resolution of 1 × 2 μm and optical section thickness of 4 μm.

A single examiner, independent of the treating physicians and not involved in clinical assessments, acquired the IVCM images. The examiner had no access to clinical findings or scheduled visit information. Central corneal imaging and imaging of the same prespecified central region of the inferior bulbar conjunctiva were targeted at every visit. After topical anesthesia with 0.5% proparacaine hydrochloride (Poencaína®; POEN S.A.U., Buenos Aires, Argentina), polyacrylic acid 0.2% gel (Acrylarm®; POEN S.A.U., Argentina) was used as the coupling medium between the contact cap and ocular surface.

The procedure lasted approximately 10 min per participant. Before leaving the examination room, each participant received one prophylactic drop of moxifloxacin 0.5% ophthalmic solution (Flomox®; MaxVision, Buenos Aires, Argentina). The manufacturer's default acquisition settings were maintained throughout the study.

At least 20 frames were acquired per structure, eye, and visit. Frames eligible for quantitative analysis were required to be in focus, free of clinically relevant motion or contact artifacts, and to depict the intended tissue layer; five eligible frames per structure, eye, and visit were selected using computer-generated random numbers with the RANDBETWEEN function in Microsoft Excel, version Microsoft 365 Apps for business (Microsoft Corporation, Redmond, WA, USA). Quantitative measurements were performed semi-automatically by a different analyst in ImageJ, version 1.54 (National Institutes of Health, Bethesda, MD, USA). The analyst was not involved in image acquisition or clinical evaluation but was aware of the visit time point; therefore, image selection and quantitative analysis were not masked to visit. The image analyst was not the examiner responsible for image acquisition and remained independent of clinical evaluations. The median of the five frame-level measurements was used as the eye-level value at each visit. Stromal reflectivity was calculated from grayscale images as (mean gray value − minimum gray value)/maximum gray value, and the median of five frames was retained. Intra- and interobserver repeatability were not formally evaluated.

The participant flow for the VCM sub-study is shown in Figure 1. The images displayed in Figures 2–7 were selected after completion of the quantitative analysis on the basis of technical quality and availability of a complete matched serial set from one eye; they are illustrative and were not used to infer a cohort-level effect. Figure 8 shows qualitative nerve findings from different eyes and participants. Quantitative analysis was performed on the original unadjusted grayscale frames. Any display-level brightness or contrast adjustment was applied uniformly across panels within a figure.

The parent cohort comprised 30 participants [29]. The IVCM component, its outcomes, assessment schedule, and image-sampling procedure were prospectively specified in the protocol before enrollment of the sub-study participants, but no separate inferential statistical analysis plan or participant-level sample-size calculation was established. At the six centers participating in the parent study, one treatment kit was provided for the IVCM component. Consecutive parent-study participants were screened for eligibility for the IVCM sub-study. The additional logistical requirement referred to the participant's availability and willingness to travel from the treating physician's office to a separate designated center, where all serial IVCM examinations were performed by a masked operator. The first consecutive participant at each center who fulfilled the additional sub-study eligibility criterion of at least 24 months of prior BAK-DT exposure and agreed to serial 12-month imaging was invited. Four of the six centers identified one eligible participant each, whereas the remaining two centers did not identify any participants meeting the additional sub-study eligibility criterion during the enrollment period. Selection was not based on baseline IVCM findings or post-acquisition image quality. All four participants completed the five scheduled IVCM visits.

Because the eight eyes arose from only four participants, fellow-eye observations were considered correlated descriptive measurements rather than eight independent observations. All eight eyes provided evaluable eye-level values for every IVCM outcome at baseline and months 3, 6, 9, and 12. No normality tests, confidence-based hypothesis tests, pairwise comparisons, multiplicity adjustments, or claims of statistical significance were used in the revised analysis. Eye-level values are summarized as median (interquartile range). Case-level direction of change is described, and individual eye-level values are provided in Supplementary Table S1. Analyses were performed with IBM SPSS Statistics, version 22.0 (IBM Corp., Armonk, NY, USA).

3. Results

Four participants (eight eyes) with POAG and OSD were included. Two were women, and two were men; median age was 77.5 years (IQR, 31.75). All had received BAK-DT for at least 24 months, and all completed baseline and months 3, 6, 9, and 12 IVCM assessments (Figure 1). No participant discontinued, and no treatment-emergent non-ocular or unexpected ocular adverse event was reported.

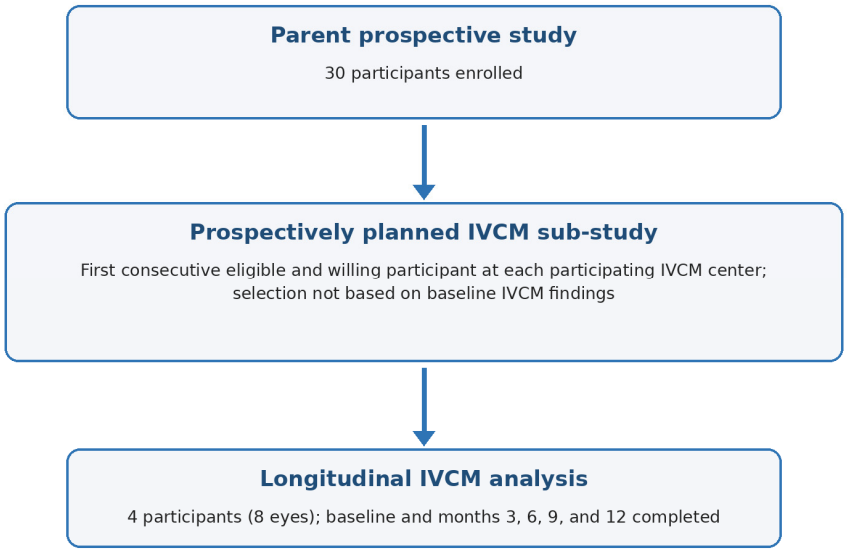


Figure 1. Participant flow from the parent study to the longitudinal IVCM pilot case series. IVCM, in vivo confocal microscopy.

Values are eye-level descriptive summaries from eight eyes of four participants and should not be interpreted as independent observations due to within-participant eye correlation. Each value represents the median of five analyzed frames. Data are presented as median (interquartile range). Cell density is expressed as cells/mm^2; nerve length as µm/mm^2; and tortuosity and reflectivity as arbitrary units (AU). No inferential statistical testing was performed. Individual eye-level values are provided in Supplementary Table S1.

At the case level, both eyes of all four participants changed in the same direction from baseline to month 12 for four outcomes: superficial corneal and conjunctival epithelial cell density increased, whereas stromal reflectivity and dendritic cell density decreased. The magnitude and intermediate course varied across cases. The remaining outcomes did not show a uniform temporal pattern. Complete individual eye-level trajectories across all five visits are provided in Supplementary Table S1.

3.1. Superficial Corneal Epithelium

At baseline and month 3, superficial corneal epithelial images showed hyperreflective cells, pyknotic nuclei, bright cell borders, and focal dark areas compatible with epithelial cell loss (Figure 2). From month 6 onward, pyknotic nuclei were not observed in the displayed serial images, and the epithelium appeared more uniform. Median superficial epithelial cell density increased from 4886.50 (1191.95) cells/mm^2 at baseline to 6896.50 (2282.50) cells/mm^2 at month 12, an absolute difference of 2010.00 cells/mm^2 (Table 1). Both eyes of each participant changed in the same direction over this interval.

Table 1. Descriptive eye-level IVCM findings across five visits.

	Baseline	Month 3	Month 6	Month 9	Month 12
Superficial corneal epithelial cell density (cells/mm^2)	4886.50 (1191.50)	5167.00 (3544.00)	5621.50 (986.00)	6500.00 (1955.50)	6896.50 (2282.50)
Basal corneal epithelial cell density (cells/mm^2)	7315.00 (516.00)	6960.50 (888.75)	7668.00 (1125.00)	8075.00 (1520.25)	9021.50 (2050.00)
Conjunctival epithelial cell density (cells/mm^2)	4496.00 (1150.00)	4948.50 (3689.50)	6551.50 (2695.50)	5883.50 (2896.25)	7757.50 (2754.75)
Stromal reflectivity (AU)	0.27 (0.05)	0.25 (0.07)	0.20 (0.05)	0.20 (0.06)	0.20 (0.03)
Endothelial cell density (cells/mm^2)	3660.50 (895.25)	4160.00 (1731.00)	3056.50 (600.75)	3372.00 (843.25)	3783.00 (1151.00)
Dendritic cell density (cells/mm^2)	121.25 (41.88)	94.38 (53.44)	68.13 (40.94)	41.26 (27.71)	45.76 (19.77)
Sub-basal nerve length (µm/mm^2)	6267.70 (4174.64)	8038.95 (3890.72)	5908.87 (2694.79)	7550.98 (4335.75)	8418.87 (4895.18)
Nerve tortuosity (AU)	2.00 (0.00)	2.00 (0.75)	2.00 (0.00)	2.00 (0.75)	2.00 (0.75)

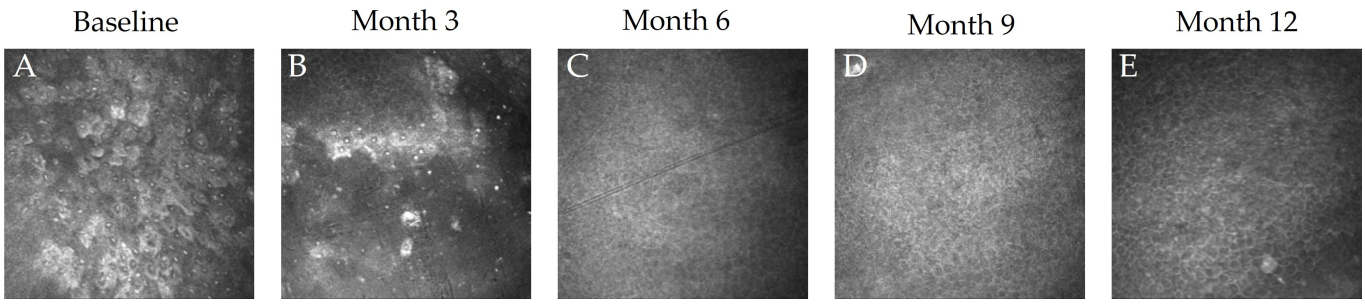


Figure 2. Serial IVCM images of the superficial corneal epithelium at baseline (**A**) and months 3, 6, 9, and 12 (**B**–**E**). Each frame represents a 400 × 400 µm field. All panels derive from the same eye of one participant and are illustrative rather than cohort-representative.

3.2. Basal Corneal Epithelium

Basal corneal epithelial cells showed dark cytoplasm and clearly distinguishable bright borders. Cell density was 7315 (516) cells/mm^2 at baseline and 9021.50 (2050.00) cells/mm^2 at month 12, with fluctuation at intermediate visits (Figure 3 and Table 1). No inferential interpretation was assigned to this descriptive pattern.

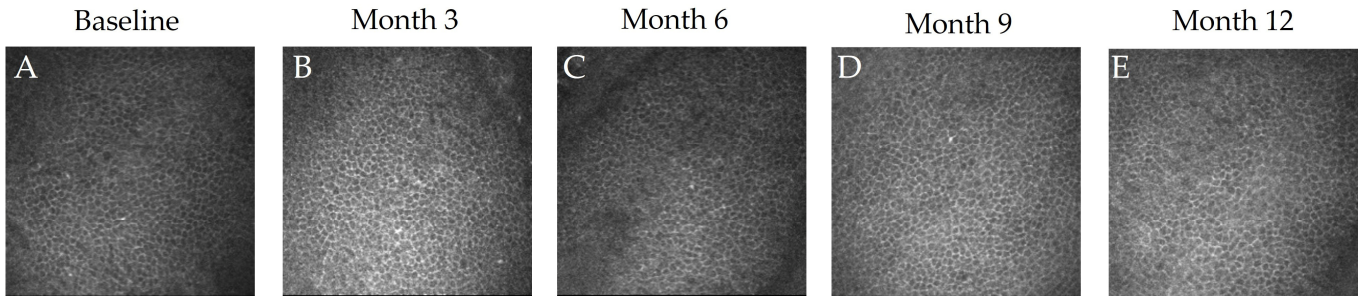


Figure 3. Serial IVCM images of the basal corneal epithelium at baseline (**A**) and months 3, 6, 9, and 12 (**B**–**E**). Each frame represents a 400 × 400 µm field. All panels derive from the same eye of one participant and are illustrative rather than cohort-representative.

3.3. Superficial Conjunctival Epithelium

At baseline, the displayed superficial conjunctival epithelial image showed elongated hyperreflective cells, non-polygonal morphology, bright borders, and pyknotic nuclei (Figure 4). Goblet cells were visible in the displayed images from month 6 onward, while pyknotic nuclei and hyperreflective borders appeared less prominent. Median conjunctival epithelial cell density increased from 4496.00 (1150.00) cells/mm^2 at baseline to 7757.50 (2754.75) cells/mm^2 at month 12, an absolute difference of 3261.5 cells/mm^2 (Table 1). Both eyes of each participant changed in the same direction over this interval.

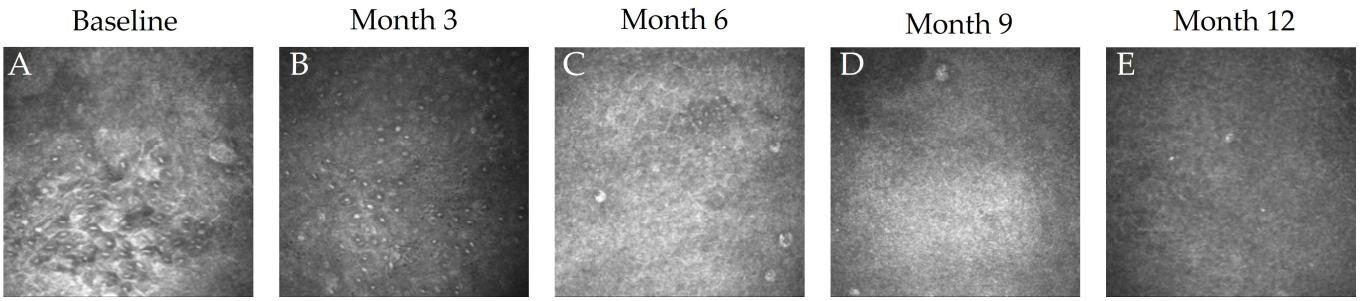


Figure 4. Serial IVCM images of the superficial conjunctival epithelium at baseline (**A**) and months 3, 6, 9, and 12 (**B**–**E**). Each frame represents a 400 × 400 µm field. All panels derive from the same eye of one participant and are illustrative rather than cohort-representative.

3.4. Stroma

At baseline, the displayed posterior stromal image showed hyperreflective keratocytes and increased background reflectivity (Figure 5). Reflectivity appeared lower across the serial display. Median stromal reflectivity decreased from 0.27 (0.05) AU at baseline to 0.20 (0.07) AU at month 12, an absolute difference of −0.07 AU (Table 1). Both eyes of each participant changed in the same direction over this interval; this imaging measure does not directly demonstrate collagen realignment or extracellular-matrix reorganization.

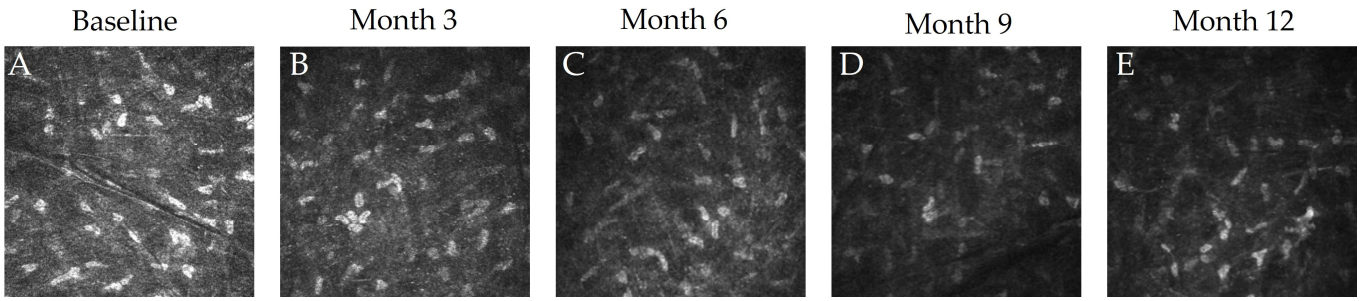


Figure 5. Serial IVCM images of the posterior corneal stroma at baseline (**A**) and months 3, 6, 9, and 12 (**B**–**E**). Each frame represents a 400 × 400 µm field. All panels derive from the same eye of one participant and are illustrative rather than cohort-representative.

3.5. Endothelium

Endothelial images showed mild polymegathism throughout follow-up, with dark cell borders, hyperreflective cytoplasm, and focal nuclear hyperreflectivity in some cells. Median endothelial cell density fluctuated across visits without a uniform temporal pattern (Figure 6 and Table 1).

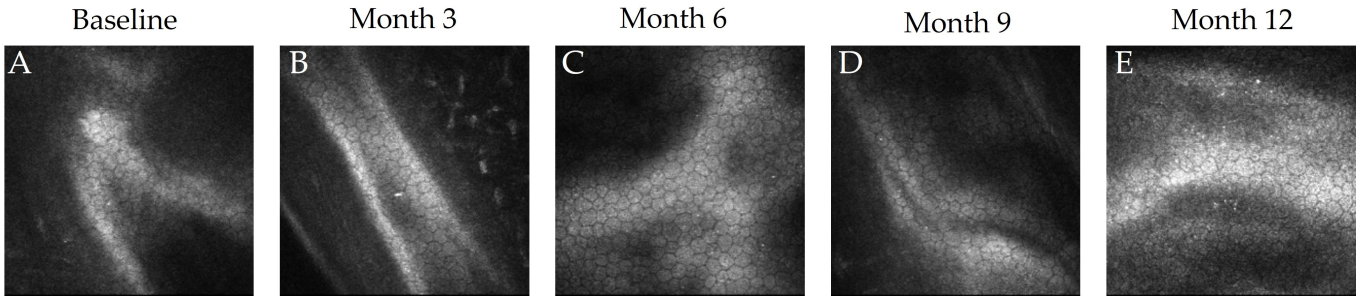


Figure 6. Serial IVCM images of the corneal endothelium at baseline (**A**) and months 3, 6, 9, and 12 (**B**–**E**). Each frame represents a 400 × 400 µm field. All panels derive from the same eye of one participant and are illustrative rather than cohort-representative.

3.6. Dendritic Cell Density

Mean dendritic cell density decreased from 121.25 (41.88) cells/mm^2 at baseline to 45.76 (19.77) cells/mm^2 at month 12, an absolute difference of −75.49 cells/mm^2 (Figure 7 and Table 1). Both eyes of each participant changed in the same direction over this interval.

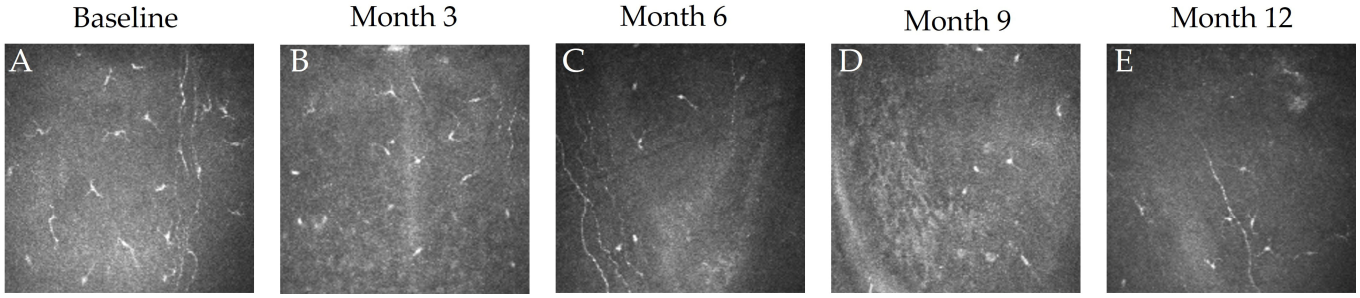


Figure 7. Serial IVCM images of corneal dendritic cells at baseline (**A**) and months 3, 6, 9, and 12 (**B**–**E**). Each frame represents a 400 × 400 µm field. All panels derive from the same eye of one participant and are illustrative rather than cohort-representative.

3.7. Corneal Sub-Basal Nerve Plexus

Median sub-basal nerve length and tortuosity fluctuated across visits without a uniform temporal pattern (Table 1). Qualitative evaluation showed interindividual variability, including thickened or thinned fibers, microneuromas, looping, bead-like formations, and pronounced tortuosity (Figure 8).

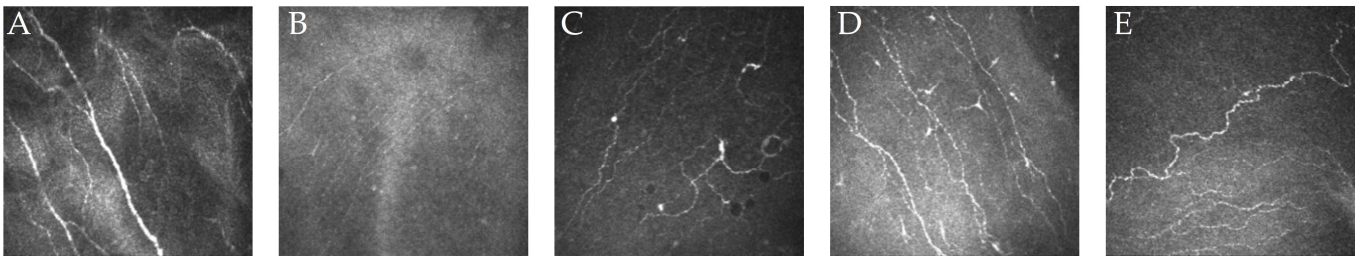


Figure 8. Qualitative IVCM examples of the sub-basal nerve plexus: thickened fibers (**A**), thinned fibers (**B**), microneuromas and looping (**C**), bead-like formations with dendritic cells (**D**), and pronounced tortuosity (**E**). Each frame represents a 400 × 400 µm field. Panels derive from different eyes and participants and are not a longitudinal series.

4. Discussion

4.1. Principal Findings and Interpretation

This descriptive pilot case series identified longitudinal IVCM changes after four participants switched from BAK-DT to PF-DT. Across all four cases and both eyes, superficial corneal and conjunctival epithelial cell density increased, and stromal reflectivity and dendritic cell density decreased between baseline and month 12. These temporal patterns are compatible with lower ocular-surface inflammatory activity, but the study design cannot establish that BAK withdrawal caused them.

The parent publication reported OSDI, tear-film staining, hyperemia, intraocular pressure, safety, tolerability, and satisfaction outcomes for the full 30-participant cohort through week 24 [29]. The present report does not duplicate or reanalyze those cohort-level clinical outcomes; it focuses on previously unpublished IVCM observations collected over 12 months in the four-participant sub-study.

Prior studies have described epithelial loss, squamous metaplasia, dendritic cell activation, goblet-cell loss, altered sub-basal nerves, and greater stromal cellular activation in patients exposed to preserved glaucoma medications [21,30–33]. The within-case longitudinal design is useful for generating hypotheses about how these features evolve after a therapeutic switch, but it does not provide a counterfactual comparison and should not be interpreted as evidence of reversibility.

4.2. Superficial Corneal and Conjunctival Epithelium

Reduced superficial corneal and bulbar conjunctival epithelial cell density has been described in dry eye disease and in glaucoma patients receiving BAK-preserved treatment [21,24,31,34]. The increases observed here occurred in the same direction in all four participants, but their magnitude varied, and there was no control group.

The displayed conjunctival series also showed goblet cells from month 6 onward. Because display images were selected for technical quality and availability of a complete matched series, this qualitative observation should not be interpreted as a cohort-level change in goblet-cell density. Taken together, the epithelial findings are compatible with reduced ocular-surface stress after the switch and warrant confirmation in larger controlled studies.

4.3. Basal Corneal Epithelium

Published basal epithelial findings are inconsistent: higher, lower, and unchanged densities have been reported across dry-eye and glaucoma populations [21,22,24,32,34]. In the present series, descriptive values fluctuated and did not show a uniform temporal pattern. No mechanistic conclusion can therefore be drawn regarding preservative withdrawal or active-drug toxicity.

4.4. Stromal Findings

Activated stromal keratocytes may appear as enlarged, highly reflective nuclei on IVCM, whereas overall stromal reflectivity is also influenced by extracellular and optical tissue properties [21,23,35–37]. Martone et al. reported greater stromal keratocyte activation in glaucoma groups receiving preservative-containing antiglaucoma medications than in preservative-free and healthy control groups [21]. Although the semiquantitative keratocyte-activation grading used in that study is not directly equivalent to the continuous stromal reflectivity measure used here, the decrease in stromal reflectivity observed over time after switching to PF-DT is compatible with reduced stromal cellular activation or inflammatory activity. This interpretation remains hypothesis-generating: IVCM reflectivity does not directly demonstrate collagen realignment, extracellular-matrix reorganization, or

reversal of tissue injury, and acquisition variability and sampling of only approximately the same central region remain alternative explanations.

4.5. Dendritic Cells and Sub-Basal Nerves

Corneal dendritic cell density is commonly increased in ocular-surface inflammation, dry eye disease, and exposure to BAK-containing drops [33,38–43]. The consistent decrease observed across the four cases is compatible with reduced ocular-surface inflammatory activity after the switch. Sub-basal nerve measures showed substantial variability and no uniform temporal pattern, which may reflect biological heterogeneity, age, prior exposure, sampling variability, and the very small number of participants.

4.6. Limitations

Several limitations should be considered. The study included only four independent participants; because both eyes were evaluated, the analyses were appropriately limited to descriptive summaries. Although the first consecutive eligible participant at each participating IVCM center was enrolled, the small sample limits generalizability. The study was uncontrolled and open-label. The protocol prospectively specified the IVCM outcomes, assessment schedule, and image-sampling procedure, including the acquisition of at least 20 frames and the random selection of five images per structure, eye, and visit; however, no formal sample-size calculation was performed for this exploratory component. Image acquisition was performed by an examiner masked to the scheduled visit time point, whereas subsequent image selection and quantification were performed by an analyst who was aware of the visit. Intra- and interobserver repeatability were not assessed, and exact longitudinal registration of the sampled area was not possible. The displayed serial images were selected for technical quality and completeness and should therefore be considered illustrative rather than cohort-representative. Because no control group was included, alternative explanations for the temporal changes cannot be excluded. Larger controlled studies with appropriate accounting for within-participant correlation, fully masked image-analysis workflows, and repeatability assessment are needed to confirm these findings.

5. Conclusions

In this exploratory IVCM pilot case series, temporal increases in superficial corneal and conjunctival epithelial cell density and decreases in stromal reflectivity and dendritic cell density were observed after switching from BAK-DT to PF-DT. These findings are compatible with lower ocular-surface inflammatory activity but remain descriptive and hypothesis-generating; they do not establish causality, mechanism, or reversal of BAK-induced injury.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijtm6030037/s1, Supplementary Table S1: Individual eye-level IVCM measurements across five visits.

Author Contributions: Conceptualization, M.S.P. and A.L.; methodology, M.S.P. and A.L.; formal analysis, G.R. and A.L.; investigation, A.S., A.B., F.C., H.F., A.P., J.F.C. and A.L.; resources, M.S.P.; data curation, G.R. and A.L.; writing—original draft preparation, M.S.P., G.R. and A.L.; writing—review and editing, M.S.P., A.F. and A.L.; visualization, G.R.; funding acquisition, M.S.P. All authors have read and agreed to the published version of the manuscript.

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Employees of the sponsoring company had no role in patient recruitment, data acquisition, or clinical assessments. Their involvement was limited to data analysis, as well as manuscript drafting and critical revision.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the research ethics committee of the Swiss Argentine Clinic and Maternity and Olivos Clinic (protocol code ANTIGLAULC-001; approval dated 1 March 2023).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: De-identified individual eye-level data underlying the reported IVCM summaries are provided in Supplementary Table S1. Additional study data are not publicly available because of privacy and ethical restrictions related to patient medical records.

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Abbreviations

The following abbreviations are used in this manuscript:

DED	Dry Eye Disease
BAK	Benzalkonium Chloride
OSDI	Ocular Surface Disease Index
BUT	Break-Up Time
GAT	Goldmann Applanation Tonometer
IOP	Intraocular Pressure
PF	Preservative-free
POAG	Primary Open-Angle Glaucoma
OSD	Ocular Surface Disease
IVCM	In Vivo Confocal Microscopy
BAK-DT	BAK-Preserved Dorzolamide 2%/Timolol 0.5%
PF-DT	Preservative-Free Dorzolamide 2%/Timolol 0.5%

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