Analysis of the effects of preservative-free tafluprost on the tear proteome

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**Abstract:** The purpose of the present study was to assess the ocular surface health status in primary open angle glaucoma (POAG) patients switching from topical application of preserved latanoprost (LT) to preservative-free tafluprost (PFT) by tear proteomic monitoring. Tear fluid of POAG patients showing dry eye symptoms, using LT and switching to PFT as well as tear fluid of healthy controls has been examined. Tear proteome dynamics was monitored over 24 weeks in a first mass spectrometric explorative analysis in a small POAG patient cohort (N = 3). Longitudinal responses of candidate proteins as well as cytokines were comparatively analyzed by microarray in a larger cohort of POAG patients (N = 16) and healthy controls (N = 15). Clinical parameters including tear breakup time (TBUT) and basal Schirmer test (BST) were recorded. Distinct post-switch level alterations could be documented in POAG tear proteins (> 1000). Cellular leakage proteins, dry eye related candidates and cytokines showed predominantly level diminishment in POAG patients and approximation to the tear protein level of healthy controls in response to PFT. Tear proteins like pyruvate kinase isozymes M1/M2 or galectin 7 displayed linear tear film level decline in POAG patients (R^2 ≥0.9; P < 0.05) distinctly converging the healthy level. Proteomic outcome fit well with improved clinical parameters, TBUT and BST. In conclusion, tear proteomic alterations indicated ocular surface recovery regarding epithelia leakage and inflammation recession. Together with improved clinical parameters the study output proposes beneficial effects of PFT glaucoma therapy.

**Keywords:** Tear proteomics, glaucoma therapy, preservatives, tafluprost

**Introduction**

Topical intraocular pressure (IOP) lowering therapy is often hampered by side effects entangling ocular surface health [1]. Thereby, glaucoma patients display a prevalence of nearly 60% for ocular surface complications [2]. Adverse effects of glaucoma therapy can be elicited by the therapeutic itself or the formulation contained preservative. However, despite differences in ocular tolerability regarding therapeutic classes, e.g. prostaglandins and β-blockers [3], no distinct differences could be documented among a group of preserved prostaglandin analogs [4]. Up to date, there is growing evidence, that predominant effects are related to preservatives due to their chemical nature and toxic features [5-8]. Ocular surface complications have been reported in glaucoma patients as a result of long-term topical use of preservative containing IOP lowering therapeutics [9-11]. Benzalkonium chloride (BAC) featuring comparatively high toxic potential among common ophthalmic preservatives [12] has been reported to display dose dependent toxicity on ocular surface epithelia [13-16]. Also, negative BAC effects on the tear film encircling tear fluid production [17] and stability [18, 19] have been documented. In confidence, ocular surface complications have been reported to go along with topical usage of BAC preserved formulations in glaucoma patients [20] linked to detergent-like features of BAC as a cationic surfactant, in terms of lipid interaction, cell membrane alteration, intracellular accumulation and modulation of protein function [21-23]. Confidently, membrane damage of cornea epithelial cells, disruption of tight junctions [24] associated with increased cornea epithelium permeability [25] have been attributed to topi-
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Tear fluid was collected from POAG and healthy control subjects by use of Schirmer strips and stored at -80°C at each visit. For proteomic investigations the right eye of the probands was chosen. Tear proteins were extracted in 300 µl Dulbecco’s phosphate buffered saline (Sigma-Aldrich Co. LLC) from strips overnight at 4°C under gentle shaking conditions. For MS analysis tear samples were desalted and concentrated by use of 3 K molecular weight cut off (MWCO) spin filters (Millipore, Billerica, USA). Protein content was determined by use of a bicinchoninic acid (BCA) assay (Pierce; Thermo Scientific, Rockford, USA). For the first part of the study time point samples regarding four time points were pooled for POAG patients (N = 3; 2 females, 1 male; 66 ± 16 years) leading to four pool samples (0, 2, 12, 24 weeks ± 1 week), whereby each patient contributed equal protein content (15 µg/subject/time point) to each pool. For MA analysis longitudinal tear samples of POAG patients (N = 16, 70 ± 10 years, 11 females, 5 males) as well as healthy controls (N = 15, 11 females, 4 males, 51 ± 10 years) were individually determined. For POAG patients five time points (0, 2, 4, 12, 24 weeks ± 1 week) and for healthy controls three time points (0, 4, 24 weeks ± 1 week) were monitored. All patients were diagnosed with POAG due to glaucomatous appearance of the optic disc (notching of the neuroretinal rim, peripapillary retinal nerve fiber defects or asymmetry between two eyes > 0.2 cup disc ratio) and visual field (two reliable, consecutive abnormal visual fields within 12 month). Moreover, only patients who are in principle controlled at baseline IOP (IOP≤22 mmHg) showing dry eye symptoms (BUT < 10 mm, TBUT < 10 s) and subjective ocular surface discomfort like burning or irritation were included in the study. All POAG patients included in the study were using LT (Xalatan, Pfizer, New York, USA) eye drops ≥6 months before switching to PFT (Taflotan sine; Santen Oy, Tampere, Finland) application. As healthy controls individuals without any ocular disorders were recruited. Informed consent was taken from all study participants before onset of the study and all protocols have been approved by the local ethics committee (No. 837.453.10 [7462]) corresponding to the 1964 Declaration of Helsinki. In the course of the study clinical parameters were recorded including tear break-up time (TBUT), basal Schirmer test (BST), superficial punctate keratitis (SPK) and visual acuity (VA).

Material and methods

Study subject tear samples

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Protein fractionation & In-gel digestion

Regarding BU LC ESI MS analysis in a first step time point pool samples were fractionated by
one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (1D SDS PAGE) using 12% Bis-Tris 10-well prepared minigels. Tear samples were reduced and mixed with NuPAGE LDS sample buffer and heated for 10 min at 90°C following fractionation at 150 V. Gels were stained using the Coomassie Brilliant Blue Kit according to the protocol of the manufacturer. Equipment and chemicals were purchased from Invitrogen (Invitrogen, Carlsbad, USA). Pool sample lanes were divided into 17 slices/lane followed by trypsin digestion using a modified protocol of Shevchenkov and coworkers [37]. Slices were minced, destained, dehydrated, soaked with 60 µl of sequencing grade modified trypsin (Promega Corporation, Madison, USA) solution [15 ng/µl] and incubated overnight at 37°C. Tryptic peptide solutions were concentrated to dryness in a SpeedVac concentrator 5301 (Eppendorf, Hamburg, Germany). Digestive peptides were resolubilized in 10 µl 0.1% trifluor acetic acid (TFA) and manually purified by C18 solid phase extraction (SPE) (ZIPTIP; Millipore, Billerica, USA) prior to MS analysis. Peptides were bound in 20 binding cycles to activated tips, washed three times with 0.1% TFA and eluted twice in elution buffer (50% acetonitrile (ACN), 0.1% TFA) using a volume of 10 µl. Each slice digest was purified twice and eluting peptides were pooled. Lyophilized peptides were stored at -20°C prior to MS analysis.

Mass spectrometry (MS)

Solubilized peptides (20 µl 0.1% TFA) of each gel slice were analyzed by use of LC ESI MS. The LC system consisted of a Rheos Allegro pump (Thermo Scientific, Rockford, USA) downscaled to capillary flow as already described with weak modifications [38, 39]. As column system a 30 × 0.5 mm BioBasic C18 precolumn connected to a 150 × 0.5 mm BioBasic C18 analytical column (Thermo Scientific, Rockford, USA) protected by an A316 online precolumn filter (Upchurch Scientific, Washington, USA) was used. 200 µl/min pump flow was downscaled to capillary flow (6.7 ± 0.03 µl/min) and the LC system was directly connected to the ESI source of a LTQ Orbitrap XL hybrid instrument (Thermo Fisher Scientific, Rockford, USA) transferring ions by use of a low flow metal needle (Thermo Fisher Scientific, Rockford, USA). Thereby, 5 µl of peptides were injected for each run by use of a PAL HTS robot (CTC Analytics, Zwingen, Switzerland) realizing duplet measurements corresponding to 50 min gradient analysis (buffers: A = 98% H2O, 1.94% ACN, 0.06% methanol, 0.05% formic acid; B = 95% ACN, 3% methanol, 2% H2O, 0.05% formic acid). The gradient was programmed as follows: 15-20% B (0-2 min), 20-60% B (2-35 min), 60-100% B (35-40 min), 100-0% B (40-45 min), 0% B (45-50 min). Counteracting carryover effects, 30 min washing runs injecting 80% ACN to the system were realized. The whole workflow was administered by XCalibur 2.0.7 SP1 (Thermo Fisher Scientific). For FT MS (Fourier Transform MS) measurements a range of 300-2000 m/z in the instruments positive mode was selected and the following parameters were adjusted: max. injection time = 50 ms (Linear ion trap = LTQ), 500 ms (FT), activation = collision induced decay (CID), normalized collision energy = 35, activation time = 30 ms, activation Q = 0.25. The top five centroid detected monoisotopic peaks (charge state = 2+, intensity > 500) were selected within an isolation width of 2 m/z for fragmentation in each scan event considering dynamic exclusion of 90 s, repeat duration of 30 s and 30000 resolution.

Protein identification, relative quantification and longitudinal analysis

MS raw data were normalized based on the total ion current (TIC) and searched in combination against the SwissProt database (SwissProt_111101, 533049 sequences, 189064225 residues) using Proteome Discoverer (version 1.1, Thermo Fisher Scientific) and MASCOT (version 2.2.07). Thereby, peaks corresponding to precursor masses between 150-2000 m/z were focused on (charge states: 1+ to 4+, signal/noise > 6). Precursor mass tolerance was adjusted 20 ppm and 0.8 Da for fragments using Homo sapiens as taxonomy. Carbamidomethyl (C) was selected as fixed modification and one missed trypsin cleavage was allowed. Resulting proteins (MASCOT ion score ≥30; P < 0.05) were grouped to an mgf file and transferred together with raw files to an in-house developed label-free quantification tool [40, 41]. Thereby, peptide peak intensities were recorded with 0.05 Da mass and 60 s retention time (RT) tolerance in MS spectra followed by TIC normalization. Summarized pep-
tide intensities corresponding to each particular protein were transferred as Excel csv file to Statistica version 10 (Statsoft, Tulsa, USA). Finally, mean protein intensities of duplet runs were used for statistical longitudinal regression analysis in Statistica version 10 (Statsoft, Tulsa, USA). Protein response was defined through significant regression model fit (P < 0.05) or R² values ≥0.9. Non-fitting proteins were transferred to an “optimal match” fold change cluster analysis using the TramineR package implemented in the R-project (R version 2.8) [42], considering proteins showing a minimum 2 fold longitudinal increase or decrease pattern graduating proteins in clusters. Furthermore, selected tear film proteins of disease relevance, in particular associated to dry eye [34], were determined under relaxed condition (R²≥0.5). A combined candidate list was used for localization and functional gene ontology (GO) analysis using Ingenuity software (Ingenuity systems Inc., Redwood City, USA).

Microarray (MA)

Antibodies (AB) against representative MS candidate proteins, dry eye protein markers and selected cytokines were commercially purchased (Abcam, Cambridge, UK; Acris Antibodies GmbH, Herford, Germany; Agrenvec, Madrid, Spain; EMD Millipore Corporation, Billerica, USA; Bethyl Laboratories Inc., Montgomery, USA). For MA slide preparation a sci-FLEXARRAYER (Scienion, Dortmund, Germany) spotting robot was used. For each AB 900 pl per spot were prespotted in triplicate onto Onycyte Avid 6.5 mm × 6.5 mm nitrocellulose slides (Grace Bio-Labs Inc., Oregon, USA) and incubated with 7.5 µg fluorescence-labeled (Alexa Fluor 647, Cy5; Thermo Fisher Scientific, Rockford, USA) tear protein from POAG patients (N = 16) or healthy controls (N = 15) to determine abundance levels of target proteins in patients and control individuals over all time points. Slides were scanned by use of a 428 array laser scanner (Affymetrix Inc., Santa Clara, USA) and spot intensities were quantified by use of ImaGene software version 5.5 (BioDiscovery, Hawthorne, USA). Median spot and median background signals were exported to Statistical and difference was calculated between both values. Negative difference values were excluded from the list, which contained triplicate AB response intensities for each subject. Mean intensities including all group subjects were calculated for each time point. Regression pattern and approximation of the POAG group to the control group were analyzed determining linear regression tendencies in the POAG group and control group. Furthermore, to evaluate the level approximation degree of protein marker candidates from POAG patients to healthy controls in the course of the study, the initial and the last time point corresponding to the POAG samples were compared to the averaged healthy control level by t-test.

A brief overview to the complete MS/MA workflow is given in Figure 1.

Results

MS analysis of the tear fluid proteome of POAG patients switching from LT to PFT

BU LC ESI MS analysis of POAG tear fluid resulted in highly qualitative TIC chromatograms (Figure 2A) leading to accurate identification of tear proteins by MS/MS analysis (Figure 2B).
Tear proteomics in glaucoma therapy

RT reproducibility was determined on average displaying CV < 1% based on a selection of reporter peaks (N = 20), which were monitored in training replicate runs (N = 6). 1039 proteins could be identified in tear fluid of POAG patients (score ≥30; P < 0.05) (Supplementary Table). The majority of identified proteins were in accordance with the literature regarding MS

Figure 2. A. Representative TIC chromatogram corresponding to BU HPLC ESI MS proteomic analysis of POAG tears. B. Exemplary MS/MS spectra corresponding to unique signal peptides leading to accurate tear protein identification. Exemplarily post-switch responding candidates are shown. (MH+, diagnostic m/z value and corresponding sequence are provided in the right box).
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Based human tear film studies [43, 44]. Thereby, characteristic abundant extracellular tear proteins including lactotransferrin, haptoglobin and zinc-α-2 glycoprotein, as well as intracellular enzymes like pyruvate kinase isozymes M1/M2 (KPYM), glutathione S-transferase P or peroxiredoxin 1 could be detected in tear fluid of glaucoma patients. In summary, 36% of tear proteins were found to respond to the therapeutic switch in POAG tears. After the therapeutic switch to PFT application predominantly protein level reduction in tear fluid of POAG patients could be observed (exemplary candidates are attributed to slope bars by arrows).

Figure 3. A. Functional analysis of tear proteins responding in the course of PFT appliance in POAG patients inferred from BU HPLC ESI MS analysis. In summary, 36% of POAG tear proteins were found to be affected encircling linear, polynomial regulated tear proteins and declined tear proteins inferred from fold change analysis. B. Primary intracellular proteins were found to respond to the therapeutic switch in POAG tears. C. Slope value illustration of linear up/downregulated tear proteins referring to BU HPLC ESI MS analysis. After the therapeutic switch to PFT application predominantly protein level reduction in tear fluid of POAG patients could be observed (exemplary candidates are attributed to slope bars by arrows).
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in Figure 4). In confidence, the fold change cluster analysis revealed numerous intracellular proteins to be differentially regulated, whereby many of them displaying longitudinal level decline, e.g. serine/threonine protein kinase SMG1, probable proline dehydrogenase 2 or cyclin dependent kinase 18. In summary, distinct proteomic alterations could be observed in the tear film of POAG patients in the course of PFT utilization. Thereby, predominantly intracellular proteins, but also cell junction proteins detected in the tear film of POAG patients were affected in their longitudinal level paradigm after the therapeutic switch. Key proteins attributed to stress response and/or apoptosis showed downward drift in the course of PFT application. Also, dry eye related tear proteins showed tendencies of level changes in progress of preservative-free therapy.

MA analysis comparing tear protein profiles of LT to PFT switching POAG patients and healthy subjects

MA analysis supported most of MS findings. Dry eye associated MA analyzed proteins were lysozyme C, aquaporine 5, mucin 6, lactotransferrin, mammaglobin B, complement C3, cystatin, S100A9, α-1-antitrypsin and lysozyme C. Furthermore, antibodies against a subset of cytokines were purchased including interleukins (IL) IL1a, IL1b, IL2, IL5, IL8, IL9, IL10, IL12, IL13, IL17a, IL23a, transforming growth factors (TGF) TGF b2, tumor necrosis factor (TNF) TNF α, monocyte chemotactic protein (MCP) MCP1/ccl2 and macrophage inflammatory protein (MIP) MIP1a/ccl3. Tested proteins were selected due to their disease relevance and association to dry eye. To validate MS find-

Table 1. Tear proteins displaying a longitudinal level response in POAG patients in the course of preservative-free tafluprost application. \(R^2\) values, \(p\)-values of regression fit and slope values of regression are provided

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ings, commercial antibodies against MS derived candidates and additional representative candidates have been selected to support proteomic level patterns including antibodies against serotransferrin, plectin, kinectin, KPYM, myosin 10, annexin A5, annexin A11, galectin 7, Zonula occludens protein (ZO) ZO 1 and cadherin 5. MA analysis could reveal descriptive linear level diminishment among selected candidate proteins in the POAG group after the therapeutic switch with distinct approximation ($R^2 \geq 0.5$) to healthy control levels in the course of the study (exemplary illustrated in Figure 5).

For example, complement C3 showed a level decrease ($R^2 = 0.5442$) inferred from MS results, which could be supported by a declining convergence ($R^2 = 0.6004$) to the healthy stage by MA experiments. Numerous analyzed tear protein candidates showed a clear post-switch approximation from POAG to healthy control level, indicated by a significant level difference at study onset and a level conformation to control at the end of the study ($t$-test, $p$-values are denoted in brackets; $p_1 = \text{POAG}_{\text{timepoint1}}$ vs. Control mean level, $p_2 = \text{POAG}_{\text{timepoint5}}$ vs. Control mean level; Figure 6). Thereby, the majority of candidate proteins derived from MS analysis could be supported by the targeted MA results. KPYM showed a distinct linear level decline drawing near the control level (Figure 5), whereby this response could additionally be evaluated as a significant approximation ($p_1 = 0.0012$, $p_2 = 0.0013$).
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Figure 5. Targeted longitudinal MA analysis of tear fluid proteomic patterns of POAG patients and healthy control subjects. Mean intensities are exemplarily illustrated for two MS candidates (KPYM) and one cytokine (TGF-β2) displaying distinct approximation from the POAG protein level to the healthy control level over time.

Figure 6. Exemplary tear candidates showing significant post-switch approximation from POAG to the healthy stage comparing start point (G_T1 = POAG, 0 weeks) to study endpoint (G_T5 = POAG, 24 weeks) referring to MA analysis. Whereas, at study onset a significant difference (P < 0.05, t-test) between POAG and control levels has been observed for candidates, a significant difference could not been documented at the end of the study indicating ocular surface recovery.

p2 = 0.0740, (Figure 6). Similar responses (p1 < 0.05, p2 > 0.05) could be observed for annexin A11, kinectin and serotransferrin supporting their PFT associated level reduction observed in MS analysis. For cadherin 5 and galectin 7 only tendencies of post-switch level reduction could be documented by MA analysis. Regarding dry eye markers aquaporin 5 and complement factor C3 levels were found to significantly approach the healthy stage. In contrast to MS results, longitudinal reduction of magmoglobin B levels was recorded by MA analysis in tears of switching POAG patients. Among cytokines, distinct post-switch responses could be documented. Significant level approximation to the healthy tear condition could be observed for IL1b, IL2 and IL-23a and distinct tendencies could be demonstrated for IL-1α, IL13, IL17, TGF b2 and MIP1α/ccl3. Examples are illustrated in Figure 7. In summary, MS and MA results reveal obvious proteomic changes in the tear film of POAG patients after the therapeutic LT to PFT switch. Thereby, in the course of PFT utilization, predominantly cellular proteins, apoptotic and stress associated proteins as well as several dry eye disease associated proteins and also inflammatory cytokines were found to be diminished in the tear film of POAG patients over time drawing near the healthy tear proteome condition.
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Clinical findings

TBUT was found to be significantly improved from average 8.4 s (± 4.5 s SD) to 11 s (± 3.5 s SD) in the course of PFT utilization (0 vs. 24 weeks, \( P = 0.0357 \), Tukey HSD) indicating an increase of nearly 24% (Figure 8C). Furthermore, also beneficial effects by trend could be observed for BST displaying approximately 25% elevation from a mean value of 13.4 mm (± 6.7 mm SD) to 17.8 mm (± 7.5 mm SD) (Figure 8A) and a constant to slightly decrease (~6%) of IOP to a final value of 16.8 mmHg (± 3.1 mmHg SD) (Figure 8B) in the course of the study. SPK and VA were not found to be affected.

Discussion

Improvement of clinical parameters TBUT and BST as well as IOP constancy indicates beneficial impact of the therapeutic switch from LT to PFT topical application in glaucoma patients. Post-switch ocular surface improvement and IOP reduction performance are in accordance with the findings of other research groups. Accordingly, Konstas and colleagues reported a similar 24 h IOP lowering efficiency for PFT compared to LT [45] and also Hamacher and colleagues demonstrated equivalent therapeutic performances for both, preservative-containing and free tafluprost, documenting high ocular tolerability for PFT [28]. Moreover, clinical improvement under constant IOP reduction observed in the present study was in agreement with the work of Januleviciene and coworkers comparing application of PFT with LT in glaucoma patients [46]. Nevertheless, we could not find any amendment in SPK and VA values, which is probably due to the study period not exceeding 24 weeks. In compliance with improved clinical findings, MS/MA analysis revealed correlative proteomic alterations of the tear film. Since it could be demonstrated that primarily intracellular proteins and also cell junction associated proteins, detectable in POAG tears, have been affected by the therapeutic switch, a reduction of epithelial leakage events and therefore regenerative processes of the ocular surface can be assumed [21]. Because LT contained BAC was found to disturb biological membranes, disrupt cellular contact sites [47, 48] and generate cell lytic processes including apoptosis and necrosis [49], high abundance of intracellular protein species observed by MS analysis at the start of the study most likely reflects this tissue hurtful scenario. Thereby, intracellular proteins can easily attain the tear film through leakage from damaged ocular surface epithelia [50-52]. Since leaking intracellular enzymes have been found indicative for cellular cytotoxicity [53].
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Figure 8. Clinical parameter inspection. BST (A) and TBUT (C) showed distinct improvement in POAG patients switching from LT to PFT therapy, while IOP remained constant with slight reduction at the end of the study (B).

and allow assessment of the ocular surface health status considering lytic events of epithelial cells [47, 54-56], the observed tear level declines of intracellular enzymes, e.g. KPYM indicate recession of leakage processes. Confidently, pyruvate kinase was reported as the primary leaked protein from digitonin-permeabilized cells [57] making it highly interesting to monitor preservative induced lytic processes of the ocular surface. Moreover, annexin A11, reported to be associated with apoptotic processes in the cornea [15] and to be involved in dry eye disease [58] was found to converge to the healthy tear proteomic level in the course of the study indicating ocular surface recovery. Also, dry eye associated proteins encircling galectin 7 [59, 60], serotransferrin [61] and aquaporine 5 [62] showed a declined response in direction to the healthy status. Since aquaporin 5 leakage to the tear film was attributed to lacrimal gland damage [62, 63], a post-switch level shift refers to regenerative lacrimal secretion. Interestingly, dry eye related magmaglobin B [64] has been documented to be increased in tears of glaucoma patients undergoing topical anti-glaucoma treatment for more than one year [65] and was found to be impaired towards PFT in the present study, however exclusively by MA analysis, indicating regenerative processes. Regarding cell junctions, ocular surface recovery could be supported by longitudinal diminishment of symplekin, plectin, kinectin and cadherin 5 highly indicative for restoring cell adhesion processes [66, 67]. Since the inflammatory status of the ocular surface was reported to change distinctly after the topical appliance of BAC containing medication [68] indicated by elevation of cytokines [69-71], the observed decline in tear film cytokines can be linked to tissue recovery and reversal of BAC induced immunoinflammatory processes of the ocular surface [72]. However, particular cytokine levels remained elevated after the therapeutic switch and therefore the underlying mechanism need to be addressed in future studies with longer observation periods. Confident with observed diminishment of cytokine levels the finding of reduced inflammatory ocular surface complication related proteins, e.g. complement C3 or secretoglobin family member 1D1 [38, 64, 73] supported ocular surface recovery. In summary, clinical and proteomic findings indicating improved therapeutic tolerance in the course of the study fit well with previous comparative studies on preservative-free formulations. Also, Gimenez-Gomez and coworkers reported an improvement in dry eye symptoms when switching from preservative containing prostaglandins including LT to PFT [74]. Concluding, observed improvements are consistent with reversibility of adverse effects due to preservative avoidance [7]. Regarding the occurrence of leakage proteins in the tear film, a recent study demonstrated that lipid-modifying enzymes reach the tear film through cell membrane vesicle shedding processes under ocular surface stress condition [75]. Considering tear film dynamics, long-term effects have to be examined in larger study cohorts with increasing sensitivity in extended observation periods. In summary, beneficial ocular surface effects of a preservative-free POAG therapeutic could be documented by a combinatory proteomic analysis platform are consistent with improving clinical parameters. Reviewing clinical parameters and proteomic results of this study, it can be assumed, that regarding POAG therapy, topical application of PFT lead to rearrangement of proteomic tear film components, to an abatement of inflamma-
tion and a convergence to healthy tear film condition. These findings reflect ocular surface regeneration in POAG patients after switching from a preservative-containing therapeutic to a preservative free formulation.

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Disclosure of conflict of interest

None.

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