

STUDY PROTOCOL: GLAUCO-TECH

PROTOCOL TITLE: GLAUCO-TECH – Technological and molecular evaluation of glaucoma treatment: effectiveness and safety of preservative-free Dorzolamide–Timolol–Brimonidine combination ophthalmic drops versus preservative-containing ophthalmic drops in patients with ocular hypertension or primary open-angle glaucoma, including the evaluation of the impact on the ocular surface, glaucoma progression, safety, patient-reported outcomes, and technological (educational chatbot and use of artificial intelligence in electronic medical records) and molecular (tear proteomics) sub-studies in Cali, Colombia.

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Tabla de contenidos

STUDY PROTOCOL: GLAUCO-TECH	1
4. Rationale	8
5. Problem Statement and Justification	12
6. Research Question	13
7. Hypothesis	14
8. Operational Definitions of Objectives and Outcomes	14
9. Objective	17
10. Study Type	18
12. Variables to be Measured	22
13. Study Procedures	26
14. Adverse Event Management, Suspension Criteria, and Participant Withdrawal	31
15. Statistical Methods and Analysis Plan	33
16. Ethical and Regulatory Considerations	36
17. Study Timeline	40
18. Data Management, Monitoring, and Quality Control	43
19. Communication and Dissemination of Results	45

1. Study Title

GLAUCO-TECH – Technological and Molecular Evaluation of Therapy in Glaucoma: Effectiveness and Safety of Preservative-Free Dorzolamide–Timolol–Brimonidine Combination versus Dorzolamide–Timolol–Brimonidine with Preservatives in Patients with Ocular Hypertension or Open-Angle Glaucoma, including Assessment of Ocular Surface Impact, Glaucoma Progression, Safety, Patient-Reported Outcomes, and Sub-studies on Technological (Educational Chatbot and Artificial Intelligence–Assisted Electronic Health Record) and Molecular (Tear Proteomics) Dimensions in Cali, Colombia.

2. Protocol Summary

This study is a prospective, randomized, comparative effectiveness clinical trial with a single-blind design (masked evaluator), which will evaluate the preservative-free fixed combination of Dorzolamide–Timolol–Brimonidine versus the same combination with preservatives in patients diagnosed with primary open-angle glaucoma or ocular hypertension treated in the city of Cali, Colombia.

Adult patients with a clinical indication for triple topical hypotensive therapy, currently under treatment with the preservative-containing formulation or in the process of starting or switching therapy, will be included. Participants will be assigned to one of the two treatment groups and followed longitudinally under real-world clinical practice conditions, with standardized measurements.

The primary objective of the study is to compare the impact of both formulations on ocular surface health, integrating objective and subjective measures of dry eye. Secondly, intraocular pressure control, ocular and systemic safety and tolerability, therapeutic adherence, vision-related quality of life, and signs of structural and functional glaucomatous progression will be evaluated.

Additionally, the study incorporates two sub-studies. The technological sub-study will evaluate the effect of an educational and reminder chatbot on adherence, disease knowledge, and patient self-efficacy. The molecular sub-study will explore the proteomic profile of the tear film and its

variation in response to treatment, in order to identify molecular signatures associated with tolerability, ocular surface inflammation, and clinical response.

3. Introduction

Glaucoma is a heterogeneous group of progressive optic neuropathies characterized by optic disc cupping and visual field loss, constituting the leading cause of irreversible blindness worldwide (1). The most common form is primary open-angle glaucoma (POAG). Its global prevalence in individuals over 40 years of age is approximately 3–5% (1,2). Ocular hypertension (OHT) is a critical risk factor for developing glaucoma, and the reduction of intraocular pressure (IOP) is the only proven treatment that succeeds in slowing or halting the progression of glaucomatous damage.

In fact, multiple clinical trials have demonstrated that lowering IOP decreases the risk of conversion from OHT to glaucoma and slows disease progression (3). Consequently, therapeutic guidelines focus glaucoma management on achieving significant IOP reductions through topical hypotensive drugs, laser, or surgery (4). Typically, a reduction of at least 20–30% from baseline IOP is sought to stabilize the disease (5). Nonetheless, glaucoma management in real-world clinical practice transcends isolated tension control. Long-term adherence and the ocular tolerability of treatments represent critical determinants of therapeutic success, particularly in a chronic, asymptomatic disease in its early stages that requires indefinite treatment. This aspect acquires special relevance in patients who require multiple combination regimens or triple topical therapy to reach adequate pressure targets.

In this context, a significant number of patients with glaucoma or ocular hypertension require progressive therapeutic escalation, including double or triple topical therapy regimens, to achieve and maintain individualized pressure targets (1,2). While these strategies allow for greater IOP control, they also increase chronic exposure of the ocular surface to multiple active ingredients and preservatives, which can amplify local adverse effects and compromise overall treatment tolerability. This balance between hypotensive efficacy and ocular tolerance gains particular clinical relevance in patients requiring fixed combinations or long-term intensified therapies (1,2).

It is estimated that between 50% and 60% of glaucoma patients do not adequately follow their prescribed drop regimen (6), largely due to discomfort and dry eye symptoms induced by chronic medication (2). In other words, ocular surface disease associated with glaucoma and its treatments is highly prevalent: more than 60% of patients under treatment show signs or symptoms of tear film dysfunction or dry eye (7). These symptoms include burning, a gritty sensation, mild ocular pain, and intermittent blurred vision, which can interfere with daily activities and decrease the patient's quality of life. Various studies have documented that glaucoma patients who develop dry eye disease report greater ocular discomfort, difficulty reading, and challenges with other visual tasks, scoring lower on quality-of-life questionnaires (e.g., NEI VFQ-25, OSDI) compared to those without ocular surface complications (5). The appearance of these side effects frequently leads to therapeutic non-compliance, which in turn increases the risk of optic nerve damage progression as adequate tension control is not maintained (7). In short, the challenge in glaucoma management consists not only of lowering IOP but doing so with tolerable treatments that the patient can and wants to use consistently.

The bidirectional relationship between ocular surface disease and therapeutic adherence has been widely recognized in glaucoma management (6). The presence of persistent dry eye symptoms not only deteriorates quality of life but also directly influences patient behavior toward treatment, favoring omissions, voluntary dose reduction, or unsupervised suspension of medication. In this scenario, the systematic evaluation of the ocular surface and patient-reported outcomes becomes fundamental to understanding the real-world effectiveness of hypotensive therapies in daily clinical practice conditions (6).

The fixed combination of Dorzolamide/Timolol/Brimonidine is a widely used therapy in glaucoma due to its synergistic hypotensive effect. Dorzolamide (a carbonic anhydrase inhibitor) reduces aqueous humor production, while timolol (a non-selective beta-blocker) decreases intraocular fluid secretion; together, they achieve IOP reductions comparable to those of prostaglandin analogs, with the added convenience of a single combined presentation. However, most commercial formulations of this combination include preservatives, typically benzalkonium chloride (BAK), to extend the shelf life of the eye drops and prevent bacterial contamination of the multi-dose bottle. BAK is a cationic detergent effective as an antimicrobial, but its chronic use has been linked to ocular surface toxicity: it acts by altering the tear lipid layer, possesses pro-inflammatory

properties, and induces apoptosis of conjunctival goblet cells (1,7). As a consequence, repeated exposure to BAK can cause tear film instability, chronic inflammation, and even squamous metaplasia of the conjunctival epithelium. Clinical studies have shown that glaucoma patients using BAK-containing eye drops present significantly reduced tear break-up time (often <5 seconds) and increased tear osmolarity compared to untreated individuals (4), findings consistent with an unstable tear film and dry eye. In one study, two-thirds of patients treated for glaucoma or OHT showed altered tear quality (abnormal TBUT) and half presented a tear deficit via Schirmer's test, with increased osmolarity and dry eye severity correlating with the number of drops instilled and particularly with the use of preservative-containing formulations (4). Additionally, around 30–35% of patients under treatment develop detectable superficial punctate keratitis and corneal epithelial damage related to the chronic toxicity of the eye drops (5). The frequency and intensity of these lesions increase with the use of multiple topical medications and higher cumulative exposure to BAK (5). Consequently, patients with greater ocular surface damage tend to show significantly lower adherence and worse functional visual status (8). Literature highlights that total BAK exposure, the number of anti-glaucoma drugs, and the duration of treatment are independent risk factors for developing ocular surface disease in treated glaucoma (5). In view of this, it has been proposed to minimize the burden of preservatives and topical drugs whenever possible to prevent or mitigate dry eye syndrome secondary to anti-glaucoma treatment (2,5,7).

In response to these limitations, preservative-free formulations have been developed, designed to preserve hypotensive efficacy without inducing additional damage to the ocular surface (1,7). The availability of preservative-free fixed combinations represents a significant opportunity to reduce the chronic inflammatory burden, especially in patients under intensified treatment regimens. However, available evidence regarding their clinical impact comes mostly from monotherapy studies, with limited information on their performance in more complex fixed combinations and in populations treated in Latin American contexts (2,5,7).

On the other hand, there are important ethnic and population considerations in glaucoma. People of African descent have a much higher susceptibility to POAG compared to Caucasian individuals (6). Epidemiological studies show that the prevalence of glaucoma in people of African descent is several times higher (some reports indicate 3 to 5 times higher) than in populations of European origin, with an earlier onset and a more aggressive disease course (6). In patients of African

descent, glaucoma tends to cause optic nerve damage more rapidly and at IOP levels that are not necessarily very high, suggesting the involvement of specific structural and vascular factors (1,2,6). Likewise, differences in treatment response between ethnic groups have been described: for example, some studies suggest that prostaglandin analogs might be more effective in patients of African descent, while beta-blockers may have lower relative hypotensive efficacy in this group (6,8). Similarly, filtering surgery (trabeculectomy) presents higher rates of scarring failure in patients of African descent, whereas laser procedures such as trabeculoplasty seem to achieve very favorable IOP reductions in this population (6,8). Regarding the ocular surface, Meibomian Gland Dysfunction (MGD)—the main cause of evaporative dry eye—is especially prevalent in patients with long-standing glaucoma.

It has been observed that MGD and structural loss of tarsal glands are more frequent in those under chronic anti-glaucoma treatment (1,2), which exacerbates ocular dryness. Furthermore, disparities in access and adherence to anti-glaucoma treatment across different races have been identified; for example, African American and Hispanic patients in the US present lower rates of ophthalmological service use and follow-up than white patients (2,9). These inequities lead to worse visual outcomes in racially minoritized groups.

In this context, it is particularly relevant to develop studies that integrate the clinical evaluation of the ocular surface with strategies that strengthen adherence and follow-up in real-world practice. The use of technological support tools, such as digital educational interventions and structured clinical recording systems, offers an opportunity to improve disease understanding, therapeutic compliance, and the quality of collected data. Complementarily, tear film analysis through proteomic approaches allows for the exploration of biological mechanisms associated with inflammation, ocular surface dysfunction, and treatment response, providing a translational perspective that can enrich the understanding of the impact of preservatives in glaucoma management.

4. Rationale

Over the last decade, the search for glaucoma treatments that minimize negative impacts on the ocular surface has gained prominence, given the recognized link between chronic anti-glaucoma

therapy and dry eye disease (DED). The so-called "glaucoma-dry eye duality" has been the subject of numerous recent studies (2,9). On one hand, pharmacological advances have led to the introduction of preservative-free (PF) formulations of various anti-glaucoma medications, including the PF Dorzolamide/Timolol/Brimonidine combination. It has been demonstrated that replacing BAK-containing eye drops with preservative-free alternatives significantly improves ocular surface signs and symptoms within a few weeks (7). For instance, in a study of glaucoma patients on prostaglandin monotherapy, switching to a PF formulation reduced dry eye complaints in 100% of patients and significantly improved tear break-up time and lissamine green corneal staining (10–13). Such evidence supports the clinical benefit of eliminating BAK to preserve conjunctival-corneal health without compromising intraocular pressure control.

However, most of these studies have focused on monotherapy or dual therapy regimens; there is limited information regarding the impact of preservative-free formulations on patients requiring more complex fixed combinations or triple therapy regimens, in whom the cumulative burden of active ingredients and preservatives is typically higher (12,13,16,17).

On the other hand, therapeutic compliance is recognized as a crucial factor in the real-world efficacy (effectiveness) of glaucoma treatment. Various studies have explored interventions to improve adherence, ranging from patient education and counseling to sustained-release drug delivery devices that eliminate the need for daily instillations (14). Poor adherence is usually multifactorial, but local adverse effects (irritation, red eye, dryness) are frequently cited by patients as reasons for discontinuing drops (15). In this context, attempting to objectively measure the tolerability of a medication (for example, by evaluating changes in tear osmolarity, OSDI scores, or Schirmer tests before and after switching to PF) has become an integral part of some recent clinical studies (16,17). Additionally, quality-of-life questionnaires specific to glaucoma and the ocular surface are used to quantify how the patient perceives their vision and comfort with the treatment. This focus on patient-reported outcomes (PROs) allows for the capture of subtle differences between treatments that might not be reflected by IOP alone.

In parallel, there has been growing interest in integrating digital tools and technological support strategies to address the issue of therapeutic adherence in glaucoma (18). Structured educational interventions, automated reminders, and digital monitoring systems have shown potential to

improve treatment compliance and continuity of care, particularly in chronic diseases managed in outpatient settings. However, evidence regarding their integrated implementation within glaucoma clinical trials remains limited (18).

Nonetheless, knowledge gaps persist. To the best of our knowledge, there are no randomized clinical trials directly comparing the fixed combination of Dorzolamide/Timolol/Brimonidine with preservatives versus its preservative-free version, even less so in Latin American populations. Most data regarding the benefits of PF formulations come from studies of prostaglandin analogs or beta-blockers alone, conducted in Europe, North America, or Asia (12,13,16,17). Latin America, and Colombia in particular, lacks local evidence that considers the ethnic characteristics of its glaucoma patients. Since studies of this type have never been conducted in the Colombian population, it is relevant to investigate whether differences in therapeutic response or tolerability exist. Some publications suggest there may be genetic variations in ocular receptors influencing the efficacy of certain drugs (such as beta-blockers) across different ethnicities (8), but this has not been specifically studied in our region. Furthermore, disparities in access to preservative-free eye drops—which are often more expensive and not covered by basic insurance—could result in lower-income patients being overrepresented among those suffering from BAK-induced corneal complications (1). This underscores the importance of generating local data to guide more equitable visual health policies, such as subsidy programs or the provision of PF therapies for vulnerable populations.

Additionally, the biological mechanisms underlying preservative toxicity on the ocular surface have been sparsely explored in clinical studies (19). Tear film analysis through proteomic approaches offers an opportunity to identify biomarkers associated with inflammation, epithelial damage, and tear dysfunction induced by topical treatments, as well as to explore their relationship with clinical symptoms and patient-perceived tolerability (19).

In summary, there is a growing consensus on the need to balance hypotensive efficacy with ocular safety in glaucoma treatment. Keeping intraocular pressure under control is essential to prevent blindness, but it is equally important to ensure that treatment is well-tolerated and facilitates long-term adherence. Preservatives like benzalkonium chloride (BAK), while useful for maintaining

drug stability, represent a double-edged sword by affecting the ocular surface and, indirectly, compromising therapeutic continuity.

The introduction of preservative-free formulations seeks to optimize the patient experience and reduce local adverse effects, although it is still necessary to confirm that their hypotensive effectiveness is maintained and that the risk-benefit balance is favorable under real-world clinical practice conditions.

The objective of the present study is to compare the effectiveness, safety, and tolerability of the preservative-free Dorzolamide/Timolol/Brimonidine combination versus the BAK-containing formulation in patients with open-angle glaucoma or ocular hypertension. Furthermore, possible variations in clinical outcomes associated with individual or contextual characteristics of the treated population will be explored, including demographic, environmental, and previous therapeutic exposure factors that could influence treatment response or tolerance.

Complementarily, the study incorporates a sub-analysis aimed at evaluating technological strategies to support adherence and clinical follow-up, as well as an exploratory component of tear film proteomic analysis, in order to integrate clinical, behavioral, and molecular information.

Evaluations will include not only the intraocular pressure reductions obtained with each formulation but also the incidence of ocular surface alterations, treatment adherence (via bottle counts and compliance questionnaires), symptoms, and quality of life reported by participants, as well as objective parameters such as tear osmolarity. Finally, structural and functional follow-up (visual fields and optic nerve analysis) will be performed to determine if better treatment tolerance translates into lower disease progression in the short term.

Altogether, this work will provide clinical evidence of both local and global importance: it will help determine if the preservative-free formulation offers tangible advantages for the ocular health and quality of life of Hispanic-American glaucoma patients, and if its use could translate into better adherence and, potentially, lower rates of disease progression. Furthermore, it will contribute data on how anti-glaucoma treatments differentially affect various racial groups, contributing to a more personalized ophthalmic practice and to safer, more equitable treatment strategies to prevent glaucoma-related blindness in our population (2,9,16).

5. Problem Statement and Justification

Glaucoma is the leading cause of irreversible blindness worldwide, with POAG being the most common form. POAG has a global prevalence ranging between 3% and 5% in individuals over 40 years of age (1). OHT is a critical risk factor, and reducing IOP is the only proven therapeutic approach to slow the progression of optic nerve damage (2).

However, glaucoma management is not limited solely to IOP control. Treatment adherence and adverse effects on the ocular surface represent significant clinical challenges that require urgent investigation.

This issue gains particular relevance in patients requiring intensified treatment regimens, including fixed combinations and triple topical therapy, in whom the cumulative burden of drugs and preservatives can exacerbate ocular surface damage and compromise therapeutic continuity.

The fixed combination of dorzolamide and timolol is widely used for its synergistic effect: dorzolamide inhibits carbonic anhydrase, reducing aqueous humor production, while timolol, a beta-blocker, decreases intraocular fluid secretion. Although this combination is effective, most formulations contain BAK, a preservative that extends shelf life and reduces the risk of contamination, but has been associated with chronic corneal toxicity, inflammation, and epithelial damage (3).

Up to 60% of glaucoma patients do not adequately comply with their treatment, largely due to dry eye symptoms (3). Various studies have shown that the use of eye drops containing benzalkonium chloride is associated with a decrease in tear film break-up time (TBUT < 5 seconds) and an increase in tear osmolarity, reflecting ocular surface instability, as well as a higher incidence of punctate keratitis and squamous metaplasia (4, 5). Patients with dry eye disease secondary to the use of anti-glaucoma medications report eye pain, blurred vision, and difficulty performing daily activities (3), along with lower scores on quality-of-life questionnaires such as the National Eye Institute Visual Function Questionnaire-25 (NEI-VFQ-25) and the OSDI (5).

The chronic toxicity of benzalkonium chloride not only affects the ocular surface but also reduces therapeutic adherence, increasing the risk of glaucoma progression and irreversible blindness (3).

Furthermore, economic barriers and access to preservative-free formulations may aggravate corneal complications in some patients (7). Understanding these clinical differences is fundamental to developing personalized therapeutic strategies and reducing disparities in visual health (6).

In this context, a comprehensive evaluation of the ocular surface through objective clinical parameters and patient-reported outcomes is essential to understand the real-world effectiveness of anti-glaucoma therapies in daily clinical practice.

There are no randomized studies directly comparing Dorzolamide/Timolol/Brimonidine with and without preservatives in Latin American clinical contexts, and there is a clear need to generate local evidence to guide therapeutic decisions and access policies for safer treatments.

Likewise, most available studies do not systematically integrate adherence support strategies or biological approaches to explore the underlying mechanisms of preservative-induced toxicity on the ocular surface.

This study will evaluate not only the effectiveness in intraocular pressure control but also the impact of preservatives on the ocular surface, the relationship between corneal toxicity and therapeutic adherence, and the effects on patients' quality of life, thereby contributing to a more equitable and patient-centered clinical practice.

Complementarily, the present study incorporates a technological component aimed at supporting adherence and clinical follow-up, as well as an exploratory sub-study of tear film proteomic analysis, in order to integrate clinical, behavioral, and molecular information into the evaluation of anti-glaucoma treatment.

6. Research Question

In patients with ocular hypertension or open-angle glaucoma, does treatment with the preservative-free Dorzolamide–Timolol–Brimonidine combination improve ocular surface health, adherence, and patient-reported outcomes after a 2 to 4-month follow-up, while maintaining comparable clinical effectiveness and safety compared to the preservative-containing formulation?

7. Hypothesis

Null Hypothesis (H_0):

There are no significant differences in ocular surface health, adherence, quality of life, or clinical effectiveness between patients treated with preservative-free Dorzolamide–Timolol–Brimonidine and those receiving Dorzolamide–Timolol–Brimonidine with preservatives.

Alternative Hypothesis (H_1):

Treatment with preservative-free Dorzolamide–Timolol–Brimonidine is associated with significant improvement in ocular surface parameters (dry eye signs and symptoms), higher therapeutic adherence, better quality of life (OSDI, SANDE, EQ-5D), and superior ocular safety, while maintaining comparable or better clinical effectiveness in glaucoma control and progression compared to the preservative-containing formulation.

8. Operational Definitions of Objectives and Outcomes

The operational definitions establish the measurable parameters that will allow the evaluation of the study objectives, organized into four domains: ocular surface, clinical effectiveness, safety, and patient perception. Each outcome has been defined using objective criteria, validated instruments, and specific measurement points, in accordance with Good Clinical Practices (GCP) and international guidelines for glaucoma management and ocular surface disease.

8.1. Ocular Surface (Primary Outcome)

Ocular surface health is defined as the improvement in dry eye signs and symptoms from the baseline visit to the end of follow-up. This outcome will be evaluated through the variation in scores of the OSDI (Ocular Surface Disease Index) (20) and SANDE (Symptom Assessment in Dry Eye) (21,22) questionnaires, complemented by objective indicators such as tear osmolarity (mOsm/L), tear film break-up time (TBUT, seconds), and corneal staining (Oxford scale), assessed via fluorescein biomicroscopy (Annex 1).

8.2. Clinical Effectiveness (Secondary Outcome)

Clinical effectiveness is defined as the ability of the Dorzolamide/Timolol/Brimonidine treatment

(with or without preservatives) to maintain stable intraocular pressure (IOP) control and prevent structural and functional glaucoma progression. It will be evaluated through the mean variation of IOP (mmHg) via Goldmann applanation or air (non-contact) tonometry, changes in the peripapillary retinal nerve fiber layer (RNFL) thickness and the ganglion cell complex (GCC) measured by OCT, and the Mean Deviation (MD) and Pattern Standard Deviation (PSD) indices of the automated visual field (HVF 24-2). The time to reach stable tension control—defined as the target IOP maintained for at least two consecutive visits—will also be recorded.

Component	Indicator / Variable	Method / Instrument	Unit or Parameter
1. Pressure Control	Intraocular Pressure (IOP)	Goldmann Applanation or Air (Non-contact) Tonometry	mmHg / % reduction from baseline
2. Structural Progression	RNFL and GCC thickness	OCT (Heidelberg)	µm / percentage change
3. Functional Progression	Automated Visual Field (HVF 24-2)	Humphrey Analyzer (MD, PSD)	dB / standard deviation
4. Time to stable control	Achievement and maintenance of target IOP	Clinical follow-up	Weeks / visits required

8.3. Ocular and Systemic Safety

Safety is defined as the incidence, type, and severity of ocular or systemic adverse events associated with the use of the study medications. Events such as ocular irritation, hyperemia, keratitis, epitheliopathy, bradycardia, or hypotension will be considered. Information will be collected at each visit through directed questioning and ophthalmic examination, following the ICH-E6 (R2) guidelines (23).

8.4. Patient Perception and Adherence

Patient perception is defined as the change in tolerance, adherence, and vision-related quality of life during treatment. It will be measured using the Morisky Medication Adherence Scale (MMAS-8) (24) for adherence, the NEI-VFQ-25(25) for visual quality of life, and the DEQ-5 (26) for general health (Annex 1).

8.5. Technological Intervention: Adherence and Digital Support (Technological Sub-study)

The technological intervention is defined as the use of a digital treatment support tool, consisting of an educational and reminder chatbot integrated into a daily-use messaging platform. It is designed to reinforce disease knowledge, correct medication administration, and therapeutic adherence, without providing diagnoses or individualized clinical recommendations, based on the American glaucoma guidelines and the TFOS DEWS III ocular surface guidelines (2,27–29).

Exposure to the technological intervention will be defined as the participant's active interaction with the chatbot during the follow-up period, documented through automatic usage logs. The intervention will be standardized, and its content will not vary according to the assigned pharmacological group.

Technological outcomes will be evaluated through the following indicators:

- Digital tool usage: Number of registered interactions per participant (messages sent, responses to reminders, access to educational modules).
- Digital adherence: Percentage of reminders answered or confirmed by the participant relative to the total reminders sent.
- Knowledge of the disease and treatment: Evaluated via a brief structured Ad-Hoc questionnaire applied at the beginning and end of follow-up, based on American guidelines for open-angle glaucoma, glaucoma suspect, and TFOS DEWS III dry eye, using Spanish-language KAP (Knowledge, Attitudes, and Practices) questions (Annex 2).
- Perceived self-efficacy: The patient's confidence in their ability to correctly administer the treatment, evaluated through a validated Likert scale or specific items integrated into the follow-up questionnaire (Annex 2).

8.6. Self-care Competence and Technological Performance (Technological Sub-study)

The technological sub-study evaluates the impact of a digital support intervention based on an educational and reminder chatbot aimed at reinforcing patient knowledge, correct medication administration, and therapeutic adherence.

This sub-study integrates three complementary domains:

1. Perceived Self-Efficacy: Defined as the participant's level of confidence in their ability to correctly administer and sustain anti-glaucoma treatment. This will be evaluated using the Perceived Self-Efficacy Scale for Glaucoma Treatment (EAP-G), a four-item scale with a Likert-type numerical response from 0 to 10. The total score will be the average of the items (range 0–10), where higher values indicate greater perceived self-efficacy.
2. Self-care Competence: Defined as the participant's demonstrated ability to make basic decisions regarding the correct use of anti-glaucoma treatment. This will be measured through the Chatbot-evaluated Glaucoma Self-care Competence Index (ICAG-Chat), based on solving standardized educational micro-tasks (application technique, schedule compliance, missed dose behavior, and warning signs). Each correct task earns one point (total 0–4). Evaluation occurs during the first month and at the end of the study.
3. Digital Tool Usage: Degree of interaction with the chatbot. This is a process outcome evaluated through automatic logs including total interactions, reminders sent/confirmed, response proportion, educational module access, micro-task completion, and average response time. Technological adherence will be the proportion of effective interactions relative to scheduled interactions.

Technological sub-study outcomes will be analyzed for exploratory and quality improvement purposes. Participation is voluntary, and non-use will not affect clinical care or study retention.

9. Objective

9.1. General Objective

To evaluate and compare the effectiveness and safety of the preservative-free fixed combination of dorzolamide–timolol–brimonidine versus the same combination with preservatives in patients with ocular hypertension or primary open-angle glaucoma, primarily determining its impact on the ocular surface, as well as its relationship with therapeutic adherence, quality of life, and glaucoma control and progression in the population of Cali, Colombia.

9.2. Primary Objectives

1. To evaluate and compare the improvement in dry eye symptoms and signs between both treatment groups using clinical and subjective instruments:
 - a. OSDI and SANDE questionnaires for perceived symptoms.
 - b. Measurement of tear osmolarity, tear film break-up time (TBUT), and corneal staining for objective signs.
2. To determine the impact of the formulation type on overall ocular tolerance, therapeutic adherence, and reported quality-of-life outcomes using the DEQ-5 and NEI-VFQ-25.
3. To compare local and systemic safety between the two treatments, documenting the frequency, type, and severity of adverse events.

9.3. Secondary Objectives

1. To evaluate the clinical effectiveness of both formulations in controlling intraocular pressure (IOP) and maintaining functional and structural glaucoma stability through:
 - a. Serial IOP measurements.
 - b. Automated visual field evaluation (HVF 24-2).
 - c. Quantification of retinal nerve fiber layer (RNFL) and ganglion cell complex (GCC) thickness using OCT.
2. To determine the time to reach stable tension control in each group.
3. To describe the sociodemographic and clinical characteristics of the study population.

4. To explore the relationship between ocular surface health, adherence, and IOP control, identifying predictive factors for therapeutic response and tolerability.
5. To determine the impact of treatment on therapeutic adherence and persistence, using multimodal measures including structured self-reporting and clinical records.
6. To evaluate vision- and treatment-related quality of life using validated instruments such as DEQ-5 and NEI-VFQ-25.

9.4. Technological Sub-study Objectives

1. To quantify the effect of an educational and reminder chatbot on indicators of adherence, therapeutic persistence, glaucoma knowledge, self-efficacy, and patient-reported outcomes, as a complement to the pharmacological intervention.

9.5. Molecular Sub-study Objectives

1. To characterize the proteomic profile of the tear film and its longitudinal variation in response to treatment, comparing formulations with and without preservatives.
2. To identify molecular signatures associated with the improvement or deterioration of the ocular surface, symptoms, and clinical tolerability.
3. To explore candidate tear biomarkers that may be associated with therapeutic response, risk of ocular surface events, or poor adherence, as a translational and exploratory approach.

10. Study Type

Phase IV, randomized, single-blind (masked evaluator), controlled clinical trial of effectiveness under real-world clinical practice conditions, including exploratory technological and molecular sub-studies.

11. Sample Size and Population

11.1. Sample Size

The sample size was determined using the change in the Ocular Surface Disease Index (OSDI) score as the primary outcome, given that the main objective of the study is to evaluate the impact of treatment on ocular surface health in patients with ocular hypertension or primary open-angle glaucoma treated with Dorzolamide/Timolol/Brimonidine with or without preservatives. For the calculation parameters, estimates derived from published evidence on clinically relevant changes

in OSDI and its dispersion were used, documenting average variations in OSDI change with wide standard deviations (≈ 16 – 23 points) according to baseline severity categories and global clinical response (Clinician Global Impression and Subject Global Assessment) (30). Based on these findings, a conservative approach was adopted for the standard deviation and the minimal clinically important difference (MCID).

The calculation was performed to compare two independent means (two-tailed contrast), assuming equal variances and independence between groups, with a significance level (α) of 0.05 and a statistical power ($1-\beta$) increased to 90% to reduce the risk of Type II error. The MCID was defined as a 12-point change in OSDI, and the standard deviation was set at a conservative value of 20 points for the OSDI change.

The standard formula for comparing means between two independent groups was used:

$$n = \frac{2\sigma^2(Z_{\alpha/2} + Z_{\beta})^2}{\Delta^2}$$

Con $\alpha = 0.05$ ($Z_{\alpha/2} = 1.96$), potencia del 90 % ($Z_{\beta} = 1.28$), $\Delta = 12$ y $\sigma = 20$:

$$n = \frac{2(20)^2(1.96 + 1.28)^2}{12^2} = \frac{800 \times (3.24)^2}{144} \approx \frac{800 \times 10.50}{144} \approx 58.4$$

According to this calculation, a minimum size of 59 participants per group was estimated, for a total of 118 patients, to detect a clinically relevant difference in OSDI change with 90% power.

Considering that the study will be conducted under real-world clinical practice conditions, with longitudinal follow-up and multiple ocular surface evaluations, an additional margin was incorporated to compensate for potential loss to follow-up and ensure the maintenance of the planned statistical power. Consequently, the sample size was operatively adjusted to 70 patients per group, for a total of 140 participants, distributed equally between the two treatment arms.

This sample size guarantees a statistical power to detect clinically relevant differences in OSDI change and provides an adequate basis for secondary analyses of tolerability, adherence, intraocular pressure control, ocular and systemic safety, as well as for the exploratory analyses of the technological and molecular sub-studies included in the protocol.

11.1.1. Assignment to Treatment Groups

The assignment of participants to the pharmacological treatment groups will be performed using a computerized randomization sequence generated in the statistical software R version 4.3.2 (R Foundation for Statistical Computing, Vienna, Austria), using the `blockrand` package (version 1.5) for the generation of permuted blocks.

A 1:1 allocation scheme with variable block sizes (4 and 6) will be employed to minimize sequence predictability and preserve allocation concealment. The sequence generation will be performed using a predefined and documented seed, guaranteeing methodological reproducibility and statistical traceability.

The sequence will be generated exclusively by the study assistant, who will not participate in the clinical evaluation or the inclusion of participants. The complete allocation list will be held by the study pharmacy coordinator under restricted access and secure institutional storage.

Each eligible participant will be sequentially assigned a unique alphanumeric code (Study ID), which will correspond to a pharmacological kit number previously prepared according to the generated sequence. The procedure will be implemented under the principle of allocation concealment, ensuring that the investigator responsible for enrollment does not have access to the randomization list.

The allocation ratio will be 1:1 between:

- Group 1: Preservative-free (PF) Dorzolamide–Timolol–Brimonidine.
- Group 2: Preservative-containing Dorzolamide–Timolol–Brimonidine.

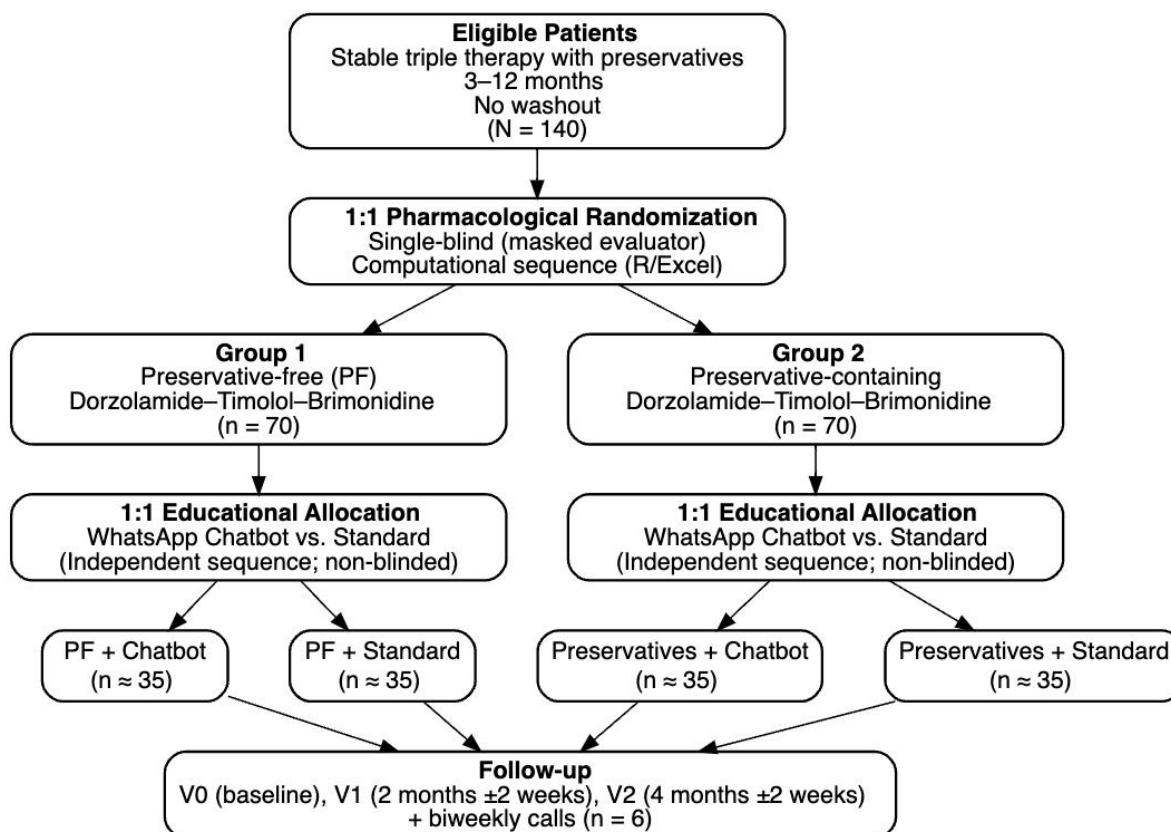
The assignment to the educational component (chatbot versus standard follow-up) will be performed independently through a second computerized sequence generated in R version 4.3.2, also using the `blockrand` package, with 1:1 permuted blocks and stratification by pharmacological group, ensuring balance among the four subgroups of the factorial design.

Given the behavioral nature of the technological intervention, this assignment will not be blinded; however, it will not modify the pharmacological treatment nor interfere with the clinical blinding of the trial.

Included patients will not suspend their previous treatment nor undergo a washout period, as the objective of the study is to compare the effectiveness and tolerability of the treatment under real-world clinical conditions of continuous use. Patients will be eligible if they are under continuous and stable treatment with the preservative-containing Dorzolamide–Timolol–Brimonidine combination for a minimum period of 3 months and a maximum of 12 months prior to inclusion, with documented intraocular pressure control and no recent modifications to the therapeutic regimen.

This pragmatic factorial design allows for the simultaneous evaluation of the impact of the pharmacological formulation type (preservative-containing versus preservative-free) and digital educational support on the ocular surface, therapeutic adherence, clinical effectiveness, and safety, without altering the standard practice of glaucoma or ocular hypertension management, and while maintaining the internal validity of the study (Figure 1).

Figure 1. Assignment Diagram and Factorial Design of the GLAUCO-TECH Stu



The figure illustrates the GLAUCO-TECH study design and participant allocation flow. A total of 140 eligible patients, under stable treatment with triple hypotensive therapy containing preservatives for 3 to 12 months and without a washout period, are included in the study. Participants initially undergo a 1:1 pharmacological randomization, single-blind with a masked evaluator, using a computational sequence to receive either a preservative-free (PF) or a preservative-containing fixed combination of Dorzolamide–Timolol–Brimonidine, with 70 patients assigned to each group.

Independently and following the pharmacological assignment, each group is further divided through a 1:1 non-blinded educational allocation to receive either a structured education program via WhatsApp chatbot or standard follow-up. This results in four approximately equivalent subgroups (n≈35 each): PF + Chatbot, PF + Standard, Preservatives + Chatbot, and Preservatives + Standard.

All participants are followed longitudinally with evaluations at the baseline visit (V0), at 2 months (V1, ± 2 weeks), and at 4 months (V2, ± 2 weeks), complemented by biweekly follow-up calls. The pragmatic factorial design allows for the simultaneous evaluation of the effect of the pharmacological formulation type and digital educational support on the ocular surface, therapeutic adherence, clinical effectiveness, and safety, while maintaining the masking of the clinical evaluator regarding the pharmacological assignment.

11.2. Population

11.2.1. Inclusion Criteria Patients who meet all of the following criteria will be included:

- Age 18 years or older.

- Diagnosis of primary open-angle glaucoma or ocular hypertension, confirmed by the treating ophthalmologist.
- Continuous and stable treatment with a topical fixed combination of Dorzolamide–Timolol–Brimonidine in a preservative-containing formulation for a minimum of 3 months and a maximum of 12 months prior to study inclusion.
- No prior use of preservative-free formulations of Dorzolamide–Timolol–Brimonidine.
- Exclusive use of the fixed combination Dorzolamide–Timolol–Brimonidine, without other concomitant ocular hypotensive agents.
- Ability to comply with study visits and procedures.
- Signed written informed consent.

11.2.2. Exclusion Criteria Patients presenting with any of the following conditions will be excluded:

- History of glaucoma, retina, or cataract surgery.
- History of corneal refractive surgery (LASIK, PRK, SMILE).
- Diagnosis of secondary glaucoma (neovascular, inflammatory, traumatic, or other secondary causes).
- History of prior procedures such as iridotomy, iridoplasty, or trabeculoplasty.
- Presence of concomitant ocular diseases that may interfere with ocular surface evaluation (e.g., Sjögren's syndrome, active infectious keratitis, or severe ocular allergy).
- Decompensated systemic conditions that contraindicate the use of topical beta-blockers (uncontrolled asthma, bradycardia, second or third-degree AV block).
- Pregnancy or breastfeeding.
- Any medical or ophthalmological condition that, in the investigator's judgment, compromises participant safety or data validity.

12. Variables to be Measured

12.1. Sociodemographic Variables

The study includes sociodemographic, clinical, ophthalmological, and adherence variables, defined and measured according to international standards in clinical ophthalmic research. Variables are collected in the electronic Case Report Form (eCRF) (Annex 2) with standardized fields and automatic validations to minimize data entry errors.

Variable	Variable Type	Categories / Unit of Measure
Sex	Nominal categorical	Male / Female / Other
Age	Continuous numerical	Years completed
Educational Level	Ordinal categorical	Primary / Secondary / Technical / University / Postgraduate
Socioeconomic Status	Ordinal categorical	Strata 1 to 6 (Local classification)

Healthcare System Affiliation	Nominal categorical	Contributory / Subsidized / Private / Uninsured
Occupation	Nominal categorical	Employed / Independent / Retired / Unemployed
Systemic Comorbidities	Nominal categorical	Hypertension / Diabetes / Dyslipidemia / Others (specify)

12.2. Ophthalmological Variables

Variable	Variable Type	Categories / Unit of Measure
Visual Acuity	Continuous numerical	Snellen (LogMAR equivalent)
OSDI (Ocular Surface Disease Index)	Ordinal categorical	Normal / Mild / Moderate / Severe
SANDE (Symptom Assessment in Dry Eye)	Continuous numerical	Visual Analog Scale (0–100)
IOP (Intraocular Pressure)	Continuous numerical	mmHg (Goldmann or air, non-contact)
Tear Osmolarity	Continuous numerical	mOsm/L
Tear Break-up Time (TBUT)	Continuous numerical	Seconds
Corneal Staining (Oxford Scale)	Ordinal categorical	Grade 0–5
Visual Field (HVF 24-2)	Numerical / Categorical	MD (dB), PSD (dB), Defect pattern (scotoma yes/no)
OCT (RNFL and GCC)	Continuous numerical	Mean μm / Percentage change
Refractive Pathologies	Nominal categorical	Myopia / Hyperopia / Astigmatism / Presbyopia

12.2.1. Visual Acuity

- **Method:** Evaluation with Snellen chart at 6 meters, with conversion to LogMAR for statistical analysis.
- **Frequency:** Baseline, Month 2, and Month 4.

12.2.2. OSDI Questionnaire

- **Method:** Validated 12-item questionnaire assessing three domains (visual symptoms, ocular discomfort, and function). Scale 0–100 (higher score indicates worse function).
- **Interpretation:** 0–12 normal; 13–22 mild; 23–32 moderate; severe.
- **Frequency:** Baseline, Month 2, and Month 4.

12.2.3. SANDE Questionnaire

- **Method:** Visual analog scale (0 to 100) for frequency and intensity. A global average score is obtained.
- **Frequency:** Baseline, Month 2, and Month 4.

12.2.4. Intraocular Pressure (IOP)

- **Method:** Measured by Goldmann applanation or air (non-contact) tonometry (average of three readings). To ensure comparability, the same tonometry method will be used for a given patient throughout all study visits.
- **Frequency:** Baseline, Month 2, and Month 4.

12.2.5. Tear Osmolarity

- **Method:** TearLab® device (Model 200000REV E). Values mOsm/L or interocular differences mOsm/L are considered abnormal.
- **Frequency:** Baseline, Month 2, and Month 4.

12.2.6. Automated Visual Field

- **Method:** Humphrey Field Analyzer (Carl Zeiss 850) protocol 24-2. MD, PSD indices, and defect type are recorded.
- **Frequency:** Baseline, Month 2, and Month 4.

12.2.7. Optical Coherence Tomography (OCT)

- **Method:** DRI Triton® (Topcon) and Heidelberg SPECTRALIS for peripapillary RNFL and GCC quantification.
- **Frequency:** Baseline, Month 2, and Month 4.

Variable	Variable Type	Categories / Unit of Measure
Treatment Adherence (MMAS-8)	Ordinal categorical	High (8), Moderate (6–7), Low (<6)
Adverse Events	Nominal categorical	Type, Severity, Causal relationship
Comorbidities	Nominal categorical	Diabetes, Hypertension, Others
Quality of Life (EQ-5D)	Ordinal categorical	Index 0–1 and VAS scale (0–100)
Visual Quality of Life (NEI-VFQ-25)	Ordinal categorical	Composite score (0–100)

12.3.1. Morisky Medication Adherence Scale (MMAS-8)

- **Method:** Validated 8-item questionnaire (Yes/No). High (8), Moderate (6–7), Low (0).
- **Frequency:** Baseline, Month 2, Month 4, and each phone follow-up.

12.3.2. Adverse Events Record

- **Method:** Directed questioning and ophthalmic exam; standardized record in CRF. Events classified by severity, causality, and outcome.
- **Frequency:** All visits and follow-up calls.

12.4. Complementary Instruments

Instrument	Objective	Timing of Application
EQ-5D	Evaluate general quality of life and perceived health	Baseline and Month 4
NEI-VFQ-25	Measure vision-related quality of life	Baseline and Month 4
MMAS-8	Evaluate treatment adherence	Baseline, Month 2, Month 4, and phone follow-up
OSDI / SANDE	Evaluate dry eye symptoms and ocular comfort	Baseline, Month 2, Month 4

12.5. Technological Sub-study Variables

Variable	Variable Type	Unit / Category
Chatbot Activation	Dichotomous categorical	Yes / No
Total Interactions	Discrete numerical	Count
Interaction Type	Nominal categorical	Educational / Reminder / Symptom report
Chatbot Usage Frequency	Discrete numerical	Interactions per month
Reported Adherence (via Chatbot)	Ordinal categorical	Adequate / Partial / Inadequate
Technical Incidences	Nominal categorical	Yes / No (describe)
Glaucoma/Treatment Knowledge	Discrete numerical	Score 0–10 (brief questionnaire)

- Method: Automatic usage logs and brief knowledge questionnaire (10 items).
- Frequency: Baseline, Month 2, and Month 4.

12.6. Proteomic Sub-study Variables

El subestudio proteómico explorará biomarcadores moleculares en película lagrimal asociados a la salud de la superficie ocular, la tolerabilidad al tratamiento y la exposición a conservantes.

Variable	Variable Type	Unit / Category
Tear Production (Schirmer I)	Continuous numerical	mm
Sampled Eye	Nominal categorical	Right / Left
Sample Code	Nominal	Alphanumeric
Time since last instillation	Continuous numerical	Minutes
Sample Status	Nominal categorical	Suitable / Unsuitable

Method: Standardized Schirmer I test, including strip collection for proteomic analysis and minimum pre-analytical recording (sampled eye, time since last instillation, and sample status).

Frequency: Baseline and Month 4.

In the proteomic sub-study, the sampling unit will be a single eye per participant to avoid interocular pseudoreplication and ensure statistical independence of the samples. In patients with unilateral involvement, the affected eye will be selected. In patients with bilateral involvement, the eye that meets the predefined study eligibility criteria will be selected.

If both eyes meet the inclusion criteria, the eye with the greatest visual impairment will be selected, defined hierarchically as follows:

1. Worst Mean Deviation (MD) on automated visual field (HVF 24-2).
2. In case of a tie or absence of visual field data, the lowest average RNFL or GCC thickness as measured by OCT.
3. If a tie persists, the worst visual acuity expressed in LogMAR.

Once the index eye is selected at the baseline visit, this same eye will be the only one sampled in all subsequent visits of the proteomic sub-study.

13. Study Procedures

13.1. Implementation of Blinding

The study will be conducted under a single-blind design with a masked evaluator for its pharmacological component. Due to logistical limitations in the commercial presentation of the formulations, it is not possible to guarantee that the packaging is indistinguishable between treatment groups. Therefore, participants and the treating glaucoma specialist responsible for clinical management will not be blinded to the assigned formulation.

Blinding will apply exclusively to the ocular surface evaluating physician, who will be responsible for measuring primary and secondary outcomes related to the ocular surface, including:

- Corneal staining (Oxford scale)
- Tear break-up time (TBUT)
- Tear osmolarity
- Structured application of OSDI and SANDE

The ocular surface evaluator will not have access to the randomization sequence or information regarding the assigned formulation. The separation of functions between the treating glaucoma specialist and the surface evaluator will be maintained throughout the study to preserve internal validity. For statistical analysis, the database will be delivered with neutral coding ("Group A" and "Group B") until the definitive closure and final validation of results.

13.2. Separation of Functions

Medication dispensing will be performed by the study pharmacy coordinator. The treating glaucoma specialist will record clinical management and intraocular pressure, while the ocular surface physician will independently perform the ocular surface evaluations. No exchange of information regarding pharmacological assignment will be permitted between these two roles. Clinical evaluation forms will not include the identification of the pharmacological group.

13.3. Unblinding and Access to the Code

The allocation list and master correspondence code will be held by the pharmacy coordinator under restricted institutional access. Since the design is single-blind and does not contemplate participant blinding, no "blind-breaking" procedure will be implemented for participants.

Access to the master code by evaluating personnel or the statistical analysis team will only be permitted exceptionally due to regulatory or safety requirements, or when indispensable for interpreting pharmacovigilance findings. Any exceptional access will be documented (date, reason, responsible parties, and corrective measures) and reported to the Institutional Review Board (IRB/CEI) and the sponsor.

13.4. Verification of Blinding Maintenance

During monitoring and internal audits, adherence to the masking procedure will be reviewed:

- Cross-checks will be applied to confirm that evaluating personnel had no access to the allocation list.
- Upon study completion, an exploratory analysis of blinding maintenance will be included, evaluating whether the evaluators' accuracy in predicting treatment differs significantly from chance (Blinding Index).
- In case of accidental exposure of the evaluator to the therapeutic assignment, the event will be documented as a protocol deviation.

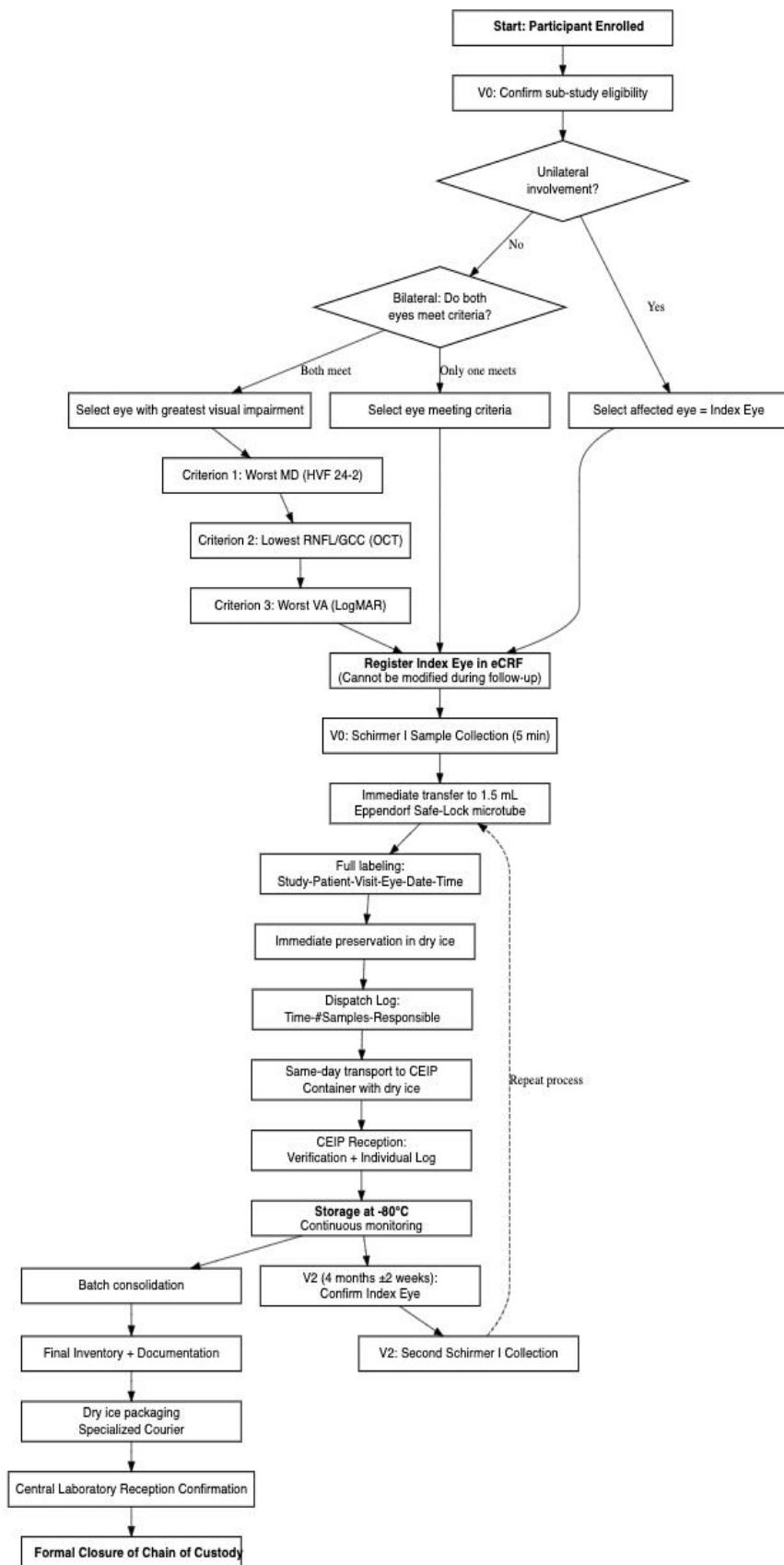
13.5. Ethical Considerations and Transparency

Evaluator masking aims to reduce observation and expectation biases. This procedure does not affect the participants' right to receive relevant health information nor interferes with standard ophthalmic care. Once the study and analysis are complete, participants may learn which formulation they were assigned to upon request.

13.6. Study Visits

Eligible participants will be randomized (1:1) and evaluated at the following visits:

- Baseline Visit (V0 – Day 0): Eligibility confirmation, signing of informed consent, collection of demographic/clinical data, randomization, delivery of assigned treatment, and initial evaluation (IOP, visual acuity, OSDI, SANDE, TBUT, osmolarity, corneal staining,



OCT, HVF, EQ-5D, NEI-VFQ-25, MMAS-8). Collection of Schirmer strip for the proteomic sub-study.

- Intermediate Visit (V1 – 2 Months 2 weeks): Re-evaluation of IOP, ocular surface (OSDI, SANDE, TBUT, osmolarity), therapeutic adherence (MMAS-8), review of adverse events, and treatment compliance.
- Final Visit (V2 – 4 Months 2 weeks): Complete evaluation of IOP, OCT (RNFL, GCC), visual field (HVF 24-2), ocular surface, adherence (MMAS-8), quality of life (EQ-5D, NEI-VFQ-25), and final adverse event recording. Collection of Schirmer strip for the proteomic sub-study.
- Remote Follow-up: Biweekly follow-up phone calls between in-person visits to reinforce adherence and monitor safety.

13.7. Clinical Monitoring

Systematic monitoring throughout the study will include:

- Serial IOP Measurement: Via Goldmann applanation or air (non-contact) tonometry at each visit, using the same method for a given patient across all visits. If a patient presents a significant IOP elevation (mmHg in two consecutive measurements), the Principal Investigator may order an additional visit or temporarily modify management.
- Comprehensive Ocular Evaluation: Visual acuity, anterior biomicroscopy, fluorescein corneal staining, and tear osmolarity at each visit.
- Adherence and Tolerance Registry: Verification of remaining medication and application of the MMAS-8 scale.
- No Washout Period: Participants will continue their previously indicated

treatment to ensure real-world clinical conditions and avoid the risk of pressure decompensation.

13.8. Study Duration

- Per Participant: 4 months. This period is sufficient to evaluate primary outcomes, as evidence shows that preservative-induced ocular surface alterations typically stabilize within 3 to 4 months of continuous use (Labbé et al., 2012; Rossi et al., 2013).
- Global Study Duration: Estimated at approximately 14 months, including recruitment, intervention, database closure, final report, and dissemination.
- Contact Points: Three in-person visits and six biweekly follow-up calls.

13.9. Proteomic Sub-study: Collection, Storage, and Processing of Tear Samples

The proteomic sub-study includes 140 participants, with two tear collection points per patient: baseline visit (V0) and final visit at 4 months (V2 2 weeks). Since a single index eye is selected per participant, the maximum total number of expected samples is 280 (140 patients $\times$ 2 visits). Each sample constitutes an independent biological unit in terms of custody, storage, and traceability, even though the patient remains the clinical unit of the study.

Tear film collection will be performed using preservative-free Schirmer I strips without anesthesia, placed in the inferior conjunctival fornix for 5 minutes following a standardized procedure. This method is the reference standard for tear collection in clinical and proteomic studies, allowing for non-invasive, reproducible, and clinically validated collection.

After collection, each strip will be immediately labeled with a unique alphanumeric code—devoid of identifiable participant data—to ensure confidentiality and traceability. Samples will be placed in sterile cryogenic tubes, preserved immediately on dry ice, and subsequently stored in an ultra-low temperature freezer (-80°C) at the CEIP (Centro de Estudios en Infectología Pediátrica) laboratory biobank (30,31).

Samples will remain stored at -80°C until processing for proteomic analysis via mass spectrometry. A documented chain of custody (date and time of collection, transfer, and storage) will be maintained at all times, in accordance with Good Clinical Practice and the center's Standard Operating Procedures (SOPs).

Protein processing and extraction from the Schirmer strips will follow validated methodological principles to minimize protein loss and maximize yield. Specifically, the sub-study design is based on the TEARDROP protocol (Tear Enrichment using Advanced Recovery via Deep Resolution of Proteins) (30), which has demonstrated superior protein recovery and expanded coverage of the tear proteome through controlled extraction and high-resolution mass spectrometry analysis. This approach allows for the identification of molecular signatures associated with ocular surface health, inflammation, and treatment tolerability.

13.11. Technological Sub-study Procedures

As part of the real-world effectiveness approach, a technological sub-study will be implemented to evaluate therapeutic adherence, patient knowledge, and the quality of clinical data collection via digital tools.

Following randomization, participants assigned to the intervention group will be activated in an educational and follow-up chatbot, which will complement standard in-person and telephone follow-up. The chatbot will provide standardized content on glaucoma, hypotensive treatment, and proper eye drop technique, as well as automated administration reminders and structured adherence reinforcement messages.

The following metrics will be automatically recorded for the intervention group: chatbot activation, total interactions, usage frequency, interaction type (educational, reminder, or symptom report), and technical incidents. The chatbot will also allow for the structured collection of self-reported adherence and adverse events, which will be validated by the research team and incorporated into the eCRF.

The control group will receive the institution's standard of care, which includes structured verbal education during the initial consultation and in-person clinical follow-up at the same intervals. No automated digital reminders or interactive tools will be implemented for this group. Data on self-reported adherence and adverse events will be collected exclusively during in-person visits through structured clinical interviews.

Patient knowledge will be evaluated in both groups using a brief 10-item questionnaire (score 0–10) at baseline, Month 2 (V1), and Month 4 (V2).

13.12. Remote Follow-up and Monitoring

To ensure therapeutic adherence and timely detection of adverse effects, a system of biweekly calls (6 contacts per patient) will be implemented by the study coordination staff. During these calls, the MMAS-8 scale will be applied, and an active search for adverse events will be conducted. Findings will be documented in the eCRF, and additional in-person visits will be scheduled if relevant symptoms are reported.

Evaluation / Activity	Baseline (V0)	2 Months (V1)	4 Months (V2)	Biweekly Calls (n=6)
Informed Consent	✓	—	—	—
Randomization	✓	—	—	—
Visual Acuity	✓	✓	✓	—
Intraocular Pressure (IOP)	✓	✓	✓	—
OSDI and SANDE	✓	✓	✓	—
TBUT and Corneal Staining	✓	✓	✓	—
Tear Osmolarity	✓	✓	✓	—
OCT (RNFL, GCC)	✓	—	✓	—
Visual Field (HVF 24-2)	✓	—	✓	—

MMAS-8 (Adherence)	✓	✓	✓	✓
DEQ-5 and NEI-VFQ-25	✓	—	✓	—
Adverse Events Registry	✓	✓	✓	✓
Schirmer for Proteomics	✓	—	✓	—
Knowledge Questionnaires (GAP/HO)	✓	✓	✓	—
Dry Eye Questionnaire	✓	✓	✓	—
EAP-G, ICAG-Chat Questionnaires	✓	✓	✓	—
Remaining Medication Review	—	✓	✓	—
Follow-up Calls (Safety/Adherence)	—	—	—	✓

14. Adverse Event Management, Suspension Criteria, and Participant

Withdrawal

14.1. General Safety Considerations

Participant safety will be monitored throughout the study through in-person evaluations and biweekly telephone follow-ups. An Adverse Event (AE) is defined as any new clinical manifestation, sign, symptom, or laboratory finding, or a significant change from the baseline state, occurring during study participation, regardless of its causal relationship with the assigned treatment.

The Principal Investigators are responsible for documenting, assessing causality and severity, and ensuring timely reporting to the Institutional Review Board (IRB/CEI) and, when applicable, the competent health authority (INVIMA), in compliance with current regulations.

14.2. Definitions

- **Adverse Event (AE):** Any untoward medical occurrence in a participant temporally associated with the use of the investigational product, whether or not considered related to the product.
- **Serious Adverse Event (SAE):** Any event that results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect, or any other medically important event according to the investigator's judgment.
- **Treatment-Related Adverse Event:** An event for which there is a reasonable possibility (possible, probable, or definite) that the investigational medicinal product caused the event.
- **Expected Adverse Event:** An event described in the summary of product characteristics or package insert for the Dorzolamide/Timolol/Brimonidine combination (e.g., hyperemia, burning, transient blurred vision, superficial keratitis, headache, bradycardia, or hypotension).

14.3. Procedure for Management and Reporting of Adverse Events

- Identification and Recording: Every AE will be documented in the Case Report Form (CRF) and the patient's medical record, including onset date, description, severity, relationship to treatment, and outcome. Decisions regarding temporary or permanent treatment suspension will be made exclusively by the treating glaucoma specialist and/or the Principal Investigator, maintaining the masking of the ocular surface evaluator.
- Severity Classification:
 - Mild: Does not interfere with daily activities.
 - Moderate: Requires intervention or symptomatic treatment.
 - Severe: Life-threatening, requires hospitalization, or results in disability.
- Clinical Management:
 - Mild AEs will be managed in an outpatient setting, using preservative-free lubricants if applicable.
 - Moderate or severe AEs will be immediately evaluated by the Principal Investigator, who may decide on temporary or definitive treatment suspension based on individual risk.
- Notification:
 - SAEs must be reported to the IRB/CEI and the sponsor within 24 hours of knowledge. Notification to INVIMA must occur within 7 calendar days for fatal or life-threatening cases, and within 15 days for all other SAEs.
 - Non-serious AEs will be included in periodic safety reports.
- Follow-up: All events will be followed until resolution or stabilization, documenting actions taken and clinical outcomes.

14.4. Criteria for Participant Suspension or Withdrawal

A participant may be withdrawn from the study for any of the following reasons:

Medical Decision:

1. Sustained IOP elevation mmHg in two consecutive measurements.
2. Development of serious or intolerable treatment-related adverse effects.
3. Significant clinical progression requiring a change in therapy.

Participant Decision:

1. Voluntary request for withdrawal (no justification required).
2. Lack of treatment adherence or repeated failure to attend scheduled visits.

Investigator or Sponsor Decision:

1. Protocol violation or data falsification.
2. Early study termination for ethical, regulatory, or safety reasons.

In all cases, withdrawal will be documented in the CRF, including the date, reason, and clinical outcome. If the participant authorizes it, final safety evaluations may be performed.

14.5. Safety Measures and Liability

The sponsor will ensure coverage through a clinical research civil liability insurance policy, protecting against potential damages derived from study participation. Policy details (insurer name, policy number, validity, and claims procedures) will be described in the Informed Consent Form. Participants will not be required to waive any legal rights or potential compensation.

15. Statistical Methods and Analysis Plan

Statistical analysis will be performed using R version 4.3.2, with a two-tailed significance level of 0.05 and 95% confidence intervals (CI). Analyses will be conducted under the modified Intention-to-Treat (mITT) and Per-Protocol (PP) principles. The primary analysis (mITT) will include all randomized participants who received at least one dose of the assigned treatment. The PP analysis will include participants who complete follow-up without major protocol deviations, according to predefined criteria.

Data will be exported directly from the electronic Case Report Form (eCRF) and subjected to cleaning, validation, and consistency control procedures. An initial exploration of missing values will be performed to verify the missingness mechanism (MCAR, MAR, or MNAR). If necessary, multiple imputation will be applied to avoid bias in the primary outcomes.

Continuous variables will be described using measures of central tendency and dispersion (mean, standard deviation, median, and interquartile range) based on the observed distribution in normality tests. Categorical variables will be summarized as absolute frequencies and percentages. To verify group balance after randomization, comparative tables of baseline characteristics will be presented using appropriate hypothesis tests based on variable type and distribution.

The database will be provided to the statistical analyst with neutral group coding ("Group A" and "Group B"), maintaining analytical blinding until database lock, consistency verification, and final freezing for analysis.

15.1. Effectiveness and Safety Analysis

The analysis will follow a mixed approach, combining bivariate comparisons and multivariate models.

- **Primary Outcome:** For the variation in the OSDI score, differences between treatment groups will be evaluated using parametric or non-parametric tests depending on data distribution. Additionally, a longitudinal repeated measures analysis will be performed to assess the evolution of the ocular surface at different follow-up points (baseline, 2 months, and 4 months), incorporating the effects of time, treatment group, and their interaction.

- **Ocular Surface Metrics:** This same model will be applied to complementary outcomes (SANDE score, tear film osmolarity, TBUT, and corneal staining), with adjustments for relevant covariates such as age, sex, duration of previous treatment, and baseline values.
- **Clinical Effectiveness (IOP):** Intraocular pressure will be analyzed throughout follow-up using repeated measures models to evaluate longitudinal differences between groups, pressure stability, and the magnitude of reduction from baseline.
- **Glaucoma Progression:** Structural progression will be studied based on changes in RNFL and GCC thickness measured by OCT, comparing intra- and inter-group means. Functional changes in MD and PSD will be considered descriptive and hypothesis-generating. Clinically significant progression will be defined by a trend analysis where the deterioration slope exceeds one standard deviation unit or a loss of more than 1 dB/year.
- **Adherence and Quality of Life:** MMAS-8 scores will be compared between groups and correlated with ocular surface indicators and pressure control. General (EQ-5D) and visual (NEI-VFQ-25) quality of life scores will be compared using analysis of covariance (ANCOVA), adjusting for potential confounders such as age, sex, glaucoma severity, and time since diagnosis.

15.2. Safety Analysis

Adverse events (AEs) will be classified by type, severity, and causality, presented as incidence rates with 95% CIs. Proportions of patients experiencing events will be compared using hypothesis tests for proportions, applying exact corrections for low counts. Serious Adverse Events (SAEs) will be described individually, detailing their clinical course, management, and outcome.

15.3. Multivariate Modeling and Bias Control

Multiple linear regression and binary logistic regression models will be constructed to identify independent predictors of ocular surface improvement, optimal pressure control, and high treatment adherence. Covariates will include age, sex, treatment duration, number of hypotensive medications, comorbidities, and baseline OSDI/MMAS-8 values.

Covariates will be selected based on clinical and statistical criteria ($p < 0.20$ in univariate analysis or recognized pathophysiological relevance). Multicollinearity and coefficient stability will be verified through internal validation procedures. Inter-observer consistency for TBUT, corneal staining, and OCT measurements will be verified using the Intraclass Correlation Coefficient (ICC).

15.4. Technological Sub-study Analysis

Variables related to the technological component—including chatbot activation, interaction logs, usage frequency, and digital engagement indicators—will be analyzed descriptively. Their association with therapeutic adherence (MMAS-8), ocular surface outcomes, and pressure control will be explored using adjusted regression models to evaluate feasibility, acceptability, and the potential modulating effect of the digital intervention.

15.5. Proteomic Sub-study Analysis

The proteomic analysis will follow a robust discovery-based pipeline to identify molecular signatures and potential biomarkers. The analysis will be performed in R, utilizing specialized Bioconductor packages to ensure reproducibility and statistical rigor.

15.7.1. Data Pre-processing and Quality Control

Raw mass spectrometry data will be processed using MaxQuant or Proteome Discoverer for protein identification and LFQ (Label-Free Quantification). Once the protein abundance matrix is exported, the following R-based steps will be implemented:

- **Data Cleaning:** Proteins identified only by site, reverse hits, or potential contaminants will be filtered out using the DEP (Differential Enrichment analysis of Proteomics) package.
- **Normalization:** To account for technical variation between LC-MS/MS runs, Variance Stabilizing Normalization (vsn) or Loess normalization will be applied.
- **Imputation:** Missing values (often representing proteins below the detection limit) will be handled using MinProb (stochastic minimal value imputation) or KNN algorithms, depending on the missingness mechanism (MAR vs. MNAR).

15.7.2. Differential Expression and Biomarker Discovery

To identify Response Markers (proteins that change significantly after treatment) and Predictive Markers (baseline proteins that explain differential tolerability):

- **Differential Abundance:** We will use the limma (Linear Models for Microarray Data) package to perform moderated t-tests. This allows for the identification of proteins that are significantly up- or down-regulated between the Preservative-Free (PF) and Preservative-containing groups.
- **Time-Series Analysis:** A paired analysis (vs.) will be conducted for each group to track the longitudinal proteomic shift.
- **Biomarker Selection:** We will employ Random Forest and LASSO (Least Absolute Shrinkage and Selection Operator) regression via the glmnet package to identify a parsimonious set of proteins (biomarker signatures) that best predict clinical outcomes such as OSDI improvement or IOP stability.

15.7.3. Dimensionality Reduction and Patient Stratification

To understand why some patients respond better or exhibit higher tolerability:

- **Unsupervised Learning:** Principal Component Analysis (PCA) and t-SNE (t-distributed Stochastic Neighbor Embedding) will be used to visualize high-dimensional proteomic clusters and identify patient "endotypes."
- **Clustering:** We will apply k-means or Hierarchical Clustering to group patients based on their tear protein profiles. This will help explain if specific baseline "molecular

signatures" of the tear film correlate with the development of treatment-induced dry eye or better adherence.

15.7.4. Pathway Analysis and Functional Annotation

To translate protein lists into biological meaning:

- Over-Representation Analysis (ORA): Using clusterProfiler, we will identify enriched Gene Ontology (GO) terms and KEGG pathways (e.g., inflammation, oxidative stress, or wound healing pathways).
- GSEA (Gene Set Enrichment Analysis): This will be used to detect subtle but coordinated changes in entire biological pathways that might be missed by single-protein analysis.
- Network Analysis: Protein-protein interaction (PPI) networks will be constructed using the STRING database and visualized in Cytoscape or the igraph R package to identify "hub proteins" that drive the ocular surface response to BAK-containing vs. PF formulations.

15.7.5. Correlation with Clinical Phenotypes

Finally, we will use Weighted Gene Co-expression Network Analysis (WGCNA) or sparse Partial Least Squares (sPLS) to integrate the proteomic data with clinical variables (Tear Osmolarity, TBUT, MMAS-8). This will help determine if specific "protein modules" in the tear film are directly responsible for the clinical perception of discomfort or the objective signs of corneal epitheliopathy.

15.6. Multiplicity and Reporting

For secondary and exploratory outcomes, p-values will be interpreted descriptively. Multiplicity adjustments (e.g., Benjamini–Hochberg FDR) will be considered for secondary analyses, while the primary outcome will remain unadjusted. Results will be presented according to CONSORT 2010 and SPIRIT 2013 guidelines.

16. Ethical and Regulatory Considerations

The present study will be conducted in strict compliance with the ethical principles governing biomedical research in human subjects, ensuring respect, dignity, safety, and the rights of all participants.

The protocol, data collection instruments, Informed Consent Forms (Annex 3), and all related documents will be submitted for review and prior approval by the Institutional Review Board (IRB/CEI) of Clínica SIGMA before any recruitment or intervention activities begin. No participant will be included without formal IRB approval.

16.1. National and International Regulatory Compliance

The study will comply with the provisions of Resolution 8430 of 1993 (Ministry of Health of Colombia), the Declaration of Helsinki (2013 revision), the CIOMS International Ethical Guidelines (2016), and the ICH-GCP E6 (R2) guidelines.

As this research involves the use of prescription drugs already approved by the national health authority and does not involve experimental phase products, it is classified as a Phase IV clinical study (post-marketing). According to Article 11 of Resolution 8430/1993, it represents minimal risk or greater than minimal controlled risk. The sponsor, Laboratorios Tecnoquímicas S.A., and the Clinical Research Center at Clínica SIGMA commit to fulfilling all responsibilities defined for investigators and sponsors under ICH-GCP and national regulations.

16.2. Informed Consent

Prior to inclusion, all patients will receive verbal and written information regarding the nature, objectives, procedures, risks, and expected benefits of the study. It will be emphasized that participation is voluntary, that subjects may withdraw at any time without negative consequences for their medical care, and that refusal to participate will not affect their access to standard treatment.

The written Informed Consent will be signed by the participant (or legal representative), the investigator obtaining the consent, and two independent witnesses. The participant will retain a signed copy. The language used will be clear and accessible, avoiding technical jargon to ensure full comprehension.

16.3. Confidentiality and Data Protection

All collected information will be treated with strict confidentiality, in accordance with Law 1581 of 2012 (Habeas Data). Data will be recorded using a unique identification code, avoiding the use of names or direct identifiers. Only the Principal Investigator and authorized personnel will have access to the key linking codes to participant identities.

Data will be stored on secure servers with restricted access for a minimum of five years following study completion, after which it will be anonymized or destroyed. The IRB, health authorities (INVIMA), and the sponsor may access anonymized data solely for auditing or monitoring purposes.

16.4. Risk and Benefit Assessment

The study involves minimal risk, as participants continue with ophthalmic treatments already approved for their indication. Potential risks are primarily limited to mild discomfort (stinging, hyperemia, transient blurred vision). The direct benefit for patients includes a detailed clinical evaluation, specialized follow-up, and the potential for a better-tolerated preservative-free formulation.

16.5. Adverse Event Management and Compensation

All adverse events (AEs) will be documented in the eCRF and reported based on severity and causality. Serious Adverse Events (SAEs) must be reported within 24 hours. The sponsor will provide coverage through a clinical research civil liability insurance policy. Policy details and claims procedures will be explicitly stated in the Informed Consent Form.

16.6. Registration and Regulatory Oversight

The study will be registered in the ClinicalTrials.gov international database prior to recruitment. INVIMA (National Health Authority) will be notified according to prescription drug research guidelines and may perform inspections at any time.

16.7. Handling, Storage, Export, and Final Disposal of Biological Samples

Tear film samples collected via Schirmer strips for the proteomic sub-study will be used exclusively for the scientific objectives defined in this protocol.

- 16.7.1. Local Storage: Samples will be stored at -80°C in certified facilities with restricted access and a documented chain of custody. The maximum local storage period is twelve (12) months.
- 16.7.2. International Export: Analysis via mass spectrometry in a specialized laboratory (USA/Europe) is contingent upon confirmed funding and formal institutional agreements. International transfer will follow cold-chain regulations for biological research materials.
- 16.7.3. Policy in Absence of Funding: If no funding or agreement is finalized within 12 months, samples will be destroyed according to institutional biohazardous waste procedures.
- 16.7.4. Prohibited Future Use: Samples will not be used for future research, secondary analysis not covered here, or commercial purposes. No permanent biobank will be established.
- 16.7.5. Final Disposal: Once analysis is complete or the storage period expires, remaining samples will be destroyed according to CEIP and current regulatory standards.

16.8. Ethical Considerations of the Technological Component

The educational chatbot is complementary and non-interventional. It does not replace medical consultation, provide diagnoses, or modify the clinical management defined by the ophthalmologist. Digital interaction data will follow the same confidentiality standards as clinical data.

16.9. Conflicts of Interest and Scientific Autonomy

The investigators declare that the study design, data collection, analysis, and publication decisions will be conducted with full scientific independence. The sponsor will have no involvement in data manipulation or the preparation of final reports. Any potential conflicts of interest will be transparently declared to the IRB and in subsequent publications.

16.10. Dissemination of Results

Results will be disseminated through scientific publications and academic presentations, ensuring participant anonymization. Publication is encouraged regardless of the direction or magnitude of the results, in accordance with the principles of transparency and scientific integrity.

17. Study Timeline

17.1. Study Phases

Phase	Main Activity	Detailed Description	Responsible Party	Estimated Duration	Period (Months)
I. Preparation & Ethics	Protocol Development & Submission	Drafting of protocol, technical annexes, informed consent, and data collection forms.	PI, Ethics Committee (IRB), Sponsor	4 weeks	Month 1
	Ethics & Regulatory Approval	IRB evaluation and INVIMA notification. Insurance policy review and regulatory compliance.	IRB – Clínica SIGMA / Sponsor	4 weeks	Month 1
	eCRF Design & Validation	Development of the electronic Case Report Form (eCRF) with quality and traceability controls.	Study Coordinator / Data Area	3 weeks	Month 1
	Team Training & Standardization	Training clinical staff on standardized procedures (IOP, TBUT, staining, eCRF, chatbot, and Schirmer collection).	Principal Investigator (PI) / Coordinator	2 weeks	Month 1
II. Recruitment	Participant Recruitment	Identifying patients in glaucoma clinics at Clínica SIGMA based on eligibility criteria.	Investigators and Clinical Optometrists	8 weeks	Months 2–3
	Randomization & Treatment Assignment	1:1 assignment via R-based computerized sequence (blockrand) with allocation concealment.	PI / Pharmacist	2 weeks	Month 3
	WhatsApp Chatbot Assignment	Parallel 1:1 assignment to the digital support program vs. standard care (independent sequence).	Study Coordinator / Data Area	2 weeks	Month 3
III. Follow-up & Safety	Baseline Evaluation (V0)	Recording of sociodemographic data, OSDI, SANDE, IOP, OCT, HVF, quality of life, and osmolarity.	Investigator / Optometrist / Research Nurse	2 weeks	Month 3

	Intermediate Visit (V1)	Adherence assessment (MMAS-8), safety, IOP, OSDI, SANDE, and osmolarity.	Investigator / Study Coordinator	4 weeks	Month 5
	Final Visit (V2)	Final effectiveness and safety evaluation: OSDI, SANDE, OCT, HVF, osmolarity, and quality of life.	Investigator / Coordinator	4 weeks	Month 7
	Technological Monitoring	Chatbot activation, interaction logs, usage frequency, and engagement metrics integrated into eCRF.	Study Coordinator / Data Area	Continuous	Months 3–7
	Proteomics Sample Collection	Coded labeling of Schirmer strips, dry ice transport, and –80 °C storage at CEIP.	Investigator / Coordinator / CEIP	8 weeks	Months 3 & 7
	Follow-up Calls	Biweekly contact for adherence monitoring and adverse event screening (6 calls per patient).	Call Center / Coordinator	Continuous	Months 3–7
	Internal Quality Monitoring	Data review, visit compliance, CRF control, and variable consistency.	Internal Monitor / Research Coordinator	12 weeks	Months 4–8
IV. Analysis & Closure	Database Lock	Validation, cleaning, and freezing of the electronic database.	Data Coordinator / Biostatistician	2 weeks	Month 9
	Statistical Analysis & Final Report	Analysis of primary/secondary outcomes, technical report, and manuscript preparation.	Biostatistician / PI	8 weeks	Months 9–11
	Regulatory Audit & Archiving	GCP compliance verification, essential document archiving, and communication with IRB/INVIMA.	Regulatory Coordinator / PI	4 weeks	Month 12
V. Dissemination	Communication of Results	Communication of global results to participants (upon request) and scientific dissemination.	Principal Investigator	3 weeks	Month 12

The execution timeline for the study "Effectiveness and safety of preservative-free Dorzolamide–Timolol–Brimonidine combination versus preservative-containing Dorzolamide–Timolol–Brimonidine in patients with ocular hypertension or open-angle glaucoma" (Protocol CEI-002) is structured as follows:

Preparation and Ethical Approval (Month 1): This initial phase focuses on the validation of the protocol, regulatory annexes, and the operational manual. Key activities include the training of the research team to ensure standardization across all clinical and technological procedures (Annex 4).

Recruitment and Randomization (Months 2–3): During this period, participants who meet the eligibility criteria will be enrolled and randomly assigned to one of the two pharmacological treatment groups (PF vs. Preservative-containing) and their respective educational support arms.

Clinical Follow-up (Months 3–7): Clinical follow-up—comprising in-person visits (V0, V1, and V2) and biweekly monitoring calls—will be conducted continuously. These activities are designed to alternate between clinical evaluation, adherence reinforcement, and safety assessments.

Safety and Quality Monitoring (Months 4–10): A cross-cutting monitoring system will be implemented throughout the study. This includes systematic eCRF reviews, data quality control, and internal audits to ensure strict compliance with Good Clinical Practice (GCP-ICH E6 R2).

Statistical Analysis (Months 7–10): This phase involves database processing, handling of missing values through imputation methods, and the multivariate analysis of primary and secondary outcomes.

Regulatory Closure and Dissemination (Months 11–12): The final stage includes the submission of the final report to the Ethics Committee (IRB) and INVIMA, the preparation of the scientific manuscript, and its submission to an international peer-reviewed journal.

In summary, this timeline outlines the logical sequence of the study, ensuring full traceability between the planning, execution, analysis, and scientific communication phases, in accordance with international clinical research standards.

Summary Timeline (Gantt Chart)

Activity / Month	1	2	3	4	5	6	7	8	9	10	11	12
Preparation & Ethics	■											
Recruitment & Randomization		■	■									
Clinical Follow-up (Visits & Calls)			■	■	■	■	■					
Safety & Quality Monitoring				■	■	■	■	■	■	■		
Statistical Analysis							■	■	■	■		
Regulatory Closure & Publication										■	■	■

18. Data Management, Monitoring, and Quality Control

Study information management will be conducted following the principles of Good Clinical Practice (ICH-GCP E6 R2) and the institutional policies of the Clínica SIGMA Clinical Research Center. Accuracy, integrity, confidentiality, and data availability will be guaranteed from initial capture to final statistical analysis.

18.1. Data Recording and Capture System

All information will be recorded in an electronic Case Report Form (eCRF), developed and managed by the research center on a secure platform. Access will be controlled via individual authentication and full traceability of every modification.

- The system will include automatic range validations, consistency checks, mandatory fields, and an audit trail documenting the date, user, and reason for every change.
- Each participant will receive a unique identification code to anonymize clinical data while maintaining the link to source files held by the Principal Investigator.
- Ophthalmological evaluations (OCT, IOP, visual field, OSDI, SANDE, DEQ-5, and NEI-VFQ-25) will be digitized and stored in encrypted repositories with restricted access.
- The eCRF system will implement differentiated access profiles to ensure the ocular surface evaluator cannot access information revealing the assigned formulation.

18.2. Information Flow and Custody

Data flow will follow a hierarchical control scheme:

1. Primary Capture: Performed by clinical or optometry staff in the eCRF during the patient visit.
2. Verification and Quality Control: Conducted by the Study Coordinator, who will review the completeness and accuracy of records weekly.
3. Internal Monitoring: Performed by the Quality Monitor, validating data consistency against clinical source documents (medical records, laboratory logs, and OCT reports).
4. Database Lock: Upon completion of follow-up for all participants and resolution of all queries, the biostatistician will authorize the database closure for analysis.

18.3. Quality Control and Data Assurance

A Quality Control Plan (QCP) will be implemented, including:

- Source Data Verification (SDV): Cross-checking between the eCRF and source documents.
- Field Supervision: Oversight of critical clinical procedures and measurements (IOP, OCT, visual field).
- Equipment Calibration: Regular control of diagnostic devices (Goldmann applanation and air/non-contact tonometer, Topcon Triton OCT, Humphrey perimeter).
- Standardized Training: For all personnel involved in data collection and entry.
- Quarterly Internal Audits: To review protocol compliance, follow-up timelines, and adverse event coding consistency.

18.4. Information Security

The data system will implement physical and digital security measures:

- Automatic daily backups on encrypted institutional servers.
- Disaster recovery protocols and redundancy in both local and private cloud storage.
- Restricted access via user profiles and hierarchical permission levels.
- SSL/TLS encryption for all communications.
- Compliance with Law 1581 of 2012 and Decree 1377 of 2013 regarding personal data protection.

18.5. Monitoring and Auditing

Monitoring will be continuous across three levels:

- Operational Monitoring: Led by the Study Coordinator, focusing on clinical data integrity.
- Internal Quality Monitoring: Conducted by the institutional research committee.
- External Monitoring: Performed by the sponsor (Laboratorios Tecnoquímicas S.A.) or a contracted entity to evaluate compliance with ICH-GCP and regulatory requirements.

18.6. Deviation Management and Version Control

Any non-compliance will be documented via a Protocol Deviation Form (PDF), classified as minor or major. Major deviations must be reported to the IRB within five business days with a corrective action plan. All document updates will be recorded in a version control table.

18.7. Final Archiving and Backup

At study closure, a Trial Master File (TMF) will be generated, including the locked final database, approved protocol, signed consents, and regulatory correspondence. This file will be held by the Clinical Research Center at Clínica SIGMA under the PI's responsibility.

19. Communication and Dissemination of Results

Results will be communicated to the IRB, INVIMA, and the sponsor through a final technical report. Participants will receive a general summary in plain language.

- Findings will be published in scientific journals and presented at congresses, adhering to ICMJE authorship criteria and ensuring the PI's academic independence.
- Only aggregated and anonymized data will be disclosed, in compliance with Law 1581 of 2012 and ICH-GCP E6 (R2).

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