Protocol Title	Clinical Study of Human Umbilical Cord Mesenchymal Stem Cell Small Extracellular Vesicle Eye Drops in the Treatment of Dry Eye Disease
Protocol No	YS245X01YK
Investigational Product	Human Mesenchymal Stem Cell Small Extracellular Vesicle Eye Drops (YS245)
Indication	Dry Eye Disease (DED)
Lead Institution	The First Affiliated Hospital of Xiamen University
Collaborator	Beijing Yisheng Medical Technology Co., Ltd.
Principal Investigators	Shiying Li Dongyan Shen
Protocol Version	V1.2
Date	20260328

Confidentiality Statement

12 Clinical Study Protocol

12.1 Study Title

Clinical Study of Human Umbilical Cord Mesenchymal Stem Cell Small Extracellular Vesicle Eye Drops in the Treatment of Dry Eye Disease

12.2 Study Objectives

To evaluate the safety and efficacy of human umbilical cord MSC-sEV eye drops for dry eye disease.

12.3 Rationale

Clinical Status and Unmet Medical Need

Currently, there is no unified classification standard for dry eye. Some international dry eye consensuses do not even establish a classification for dry eye. Considering the needs of clinical treatment and efficacy evaluation in China, and to facilitate clinical diagnosis and treatment, the "Chinese Dry Eye Expert Consensus: Definition and Classification (2020)" established three classification methods. Among them, according to the main component or functional abnormality of tears, dry eye can be classified into the following types: aqueous-deficient dry eye, lipid-deficient dry eye, mucin-deficient dry eye, tear dynamics abnormal dry eye, and mixed dry eye. The recommended treatment plans for different types of dry eye are shown in the table below.Despite available treatments, significant unmet needs remain for dry eye disease.

Table 1: Recommended Treatment Plans for Different Types of Dry Eye

Dry Eye Type	Pharmacotherapy	Physical Therapy and Others
Aqueous-deficient	Artificial tears, topical secretagogues, topical corticosteroids orchamber glasses, surgical immunosuppressants, treatment, treatment of autologous serum for severerelated systemic diseases corneal epithelial lesions	Punctal plugs, moisture
Lipid-deficient	Artificial tears, topical secretagogues, topical anti-demodex agents,treatments (including antibiotics, and/orphysical therapy, intense corticosteroids orpulsed light therapy, and immunosuppressants thermodynamic treatment)	Moisture chamber glasses, non-pharmacological eyelid
Mucin-deficient	Preservative-free or low-toxicity preservative artificial tears, topical secretagogues, topical corticosteroids and/or immunosuppressants	Punctal plugs, therapeutic contact lenses, moisture chamber glasses
Tear dynamics abnormal	Artificial tears, topical corticosteroids and/or	Therapeutic contact lenses, surgical treatment, treatment

	immunosuppressants of systemic-related diseases
Mixed type	Consider theConsider the pharmacotherapy plans fornon-pharmacological each dry eye typetreatment plans for each dry comprehensively eye type comprehensively
Aqueous-deficient	Artificial tears, topical secretagogues, topicalPunctal plugs, moisture corticosteroids orchamber glasses, surgical immunosuppressants, treatment, treatment of autologous serum for severerelated systemic diseases corneal epithelial lesions

The "Chinese Dry Eye Expert Consensus: Treatment (2020)" [12] states that the treatment principle for dry eye is to provide long-term and individualized treatment based on the type and severity of dry eye, while enabling patients to adapt to a chronic disease management system. The basic principle for selecting treatment plans is from simple to complex, from non-invasive to invasive. Treatment is divided into three aspects: etiological treatment, pharmacotherapy, and non-pharmacological treatment. Pharmacotherapy includes lubricating the ocular surface and promoting repair, anti-inflammatory treatment, and antibacterial treatment.

12.4 Expected Outcomes

To preliminarily evaluate the safety and efficacy of human mesenchymal stem cell-derived small extracellular vesicles in dry eye disease, obtain relevant clinical data to support subsequent registration studies.

12.4.1 Rationale for Using Human Mesenchymal Stem Cell-Derived Small Extracellular Vesicles for Dry Eye:

1.Biological Characteristics and Therapeutic Potential of Small Extracellular Vesicles Mesenchymal stem cell-derived small extracellular vesicles (MSC-EVs) are vesicles with a diameter of 30-150 nm, carrying bioactive molecules such as proteins (e.g., growth factors, immunomodulatory proteins), lipids, mRNA, and miRNA. They mediate intercellular communication via paracrine mechanisms. Compared to stem cell transplantation, human mesenchymal stem cell-derived small extracellular vesicles have the following advantages:

(1) Low Immunogenicity and Safety: No risk of tumorigenicity or abnormal differentiation associated with cell transplantation.

(2) Targeting and Penetration: Can cross the blood-brain barrier and blood-ocular barrier, precisely acting on the ocular surface and deep tissues.

(3) Stability: The bilayered lipid membrane protects the contents, allowing active components to be released continuously in the target area for over 72 hours.

2.Mechanism of Action of Human Mesenchymal Stem Cell-Derived Small Extracellular Vesicles in Dry Eye Disease (DED)

The core pathology of DED is ocular surface inflammation and tear film instability. Human MSC-EVs intervene through multiple pathways:

(1) Anti-inflammatory Effects: Reduce levels of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, interrupting the vicious cycle of "dryer, more inflamed."

(2) Immunomodulation: Through miRNAs (e.g., miR-204, miR-21-5p), they polarize pro-inflammatory M1 macrophages to anti-inflammatory M2 type and induce the expansion of regulatory T cells (Tregs), reshaping the ocular surface immune microenvironment.

(3) Promote Tissue Repair: Carrying growth factors such as TGF-β and BMPs, they repair lacrimal gland epithelial cells and goblet cells, increasing tear secretion by more than 30%.

3.Preclinical Research Data Support

(1) Mouse Model Validation: In a benzalkonium chloride-induced mouse dry eye model, human MSC-EVs significantly prolonged tear break-up time (TBUT), reduced corneal fluorescein staining scores (FLS), and decreased inflammatory cell infiltration.

(2) Rabbit Model Validation: In a rabbit autoimmune dry eye model, treatment with human MSC-EVs led to recovery of lacrimal gland secretory function and upregulation of anti-inflammatory cytokines such

as IL-10.

4.Clinical Trial Progress and Efficacy EvidenceRegistered Clinical Trials:

(1) A clinical trial (NCT04213248) investigating umbilical cord-derived human MSC-EV eye drops for treating graft-versus-host disease (GVHD)-related dry eye, conducted by the Zhongshan Ophthalmic Center, Sun Yat-sen University, aims to determine whether UC-MSC-EVs can alleviate dry eye symptoms in patients with cGVHD. In this single-arm study, the efficacy of UC-MSC-EVs was tested in 27 subjects aged 18 to 70 years. Subjects used artificial tears for 2 weeks, followed by 10 mg of UC-MSC-EVs four times daily for 2 weeks. The primary endpoint at the 12-week follow-up will be the change in Ocular Surface Disease Index (OSDI) score. Secondary endpoints include tear secretion, tear break-up time, fluorescein staining area, eye redness, tear meniscus height, and best-corrected visual acuity. Preliminary results show reduced ocular surface inflammation and improved tear secretion.

(2) A clinical study (NCT06543667) initiated by Iran University of Medical Sciences plans to explore the potential therapeutic effect of limbal stem cell-derived small extracellular vesicles (LSC-EVs) for refractory dry eye disease and plans to recruit 30 participants. Patients in the treatment group will receive artificial tears for 2 weeks, followed by LSC-EVs (10 μg/drop; 0.15 mL/eye/time) four times daily for 30 days. The efficacy of LSC-EV-based therapy will be assessed by measuring:

OSDI, concentration of inflammatory cytokines (IL-1, IL-6, IL-8, TNF-α, IFN-γ) in patient tears, tear secretion volume, tear break-up time, eye redness, tear meniscus height, and BCVA. Expected completion in 2025.

5.Clinical Application Prospects

Regenerative Medicine Trend: The international ophthalmology journal Acta Ophthalmologica points out that small extracellular vesicle therapy has moved from laboratory exploration to the clinical translation stage, showing significant potential, especially in the field of dry eye disease.

12.5 Study Design

Single-group, self-controlled design. All eligible subjects receive treatment and are compared with their baseline status.

12.6 Inclusion, Exclusion Criteria, and Enrollment

12.6.1 Inclusion Criteria

1)Voluntary participation and signed informed consent; willing to comply with treatment and follow-up.

2) Age ≥ 18 years, any gender

3)Best-corrected visual acuity (BCVA) ≥ 0.1 in both eyes (OU) at Visit 1 (V1).

5)History of bilateral dry eye with at least one subjective symptom: dryness, foreign body sensation, burning, fatigue, discomfort, redness, or fluctuating vision.

5)Meets one of the following at V1:

i. Positive corneal fluorescein staining, TBUT <10 s, OSDI ≥13;

ii. Negative corneal fluorescein staining, TBUT <5 s, OSDI ≥13.

12.6.2 Exclusion Criteria

1)Active ocular herpes or infection/inflammation; or history within 30 days before screening.

2)Eyelid margin abnormalities, severe conjunctivochalasis, Salzmann nodular degeneration, vitamin A deficiency, progressive pterygium, wAMD, glaucoma, DR, RVO, etc., that may increase risk or confound results.

3)Secondary ocular scarring (radiation, chemical burn, SJS, cicatricial pemphigoid, etc.) affecting compliance or evaluation.

4)Secondary Sjögren's syndrome or other autoimmune disease (RA, SLE, etc.), unless:

a. No steroids, immunomodulators, or immunosuppressants;

b. Medical condition does not affect study outcomes.

5)History of organ or bone marrow transplantation.

6)Contact lens wear within 30 days before screening.

7)Dry eye physical therapy (scrub, expression, warm compress, IPL, etc.) within 30 days.

8)Aspirin, NSAIDs, or dryness-inducing drugs (anticholinergics, SSRIs, etc.) within 30 days before dosing, unless stable ≥30 days.

9)Use of:

a. Antihistamines or any ophthalmic drugs within 14 days;

b. Artificial tears within 14 days;

c. Steroids or mast cell stabilizers within 30 days;

d. Varenicline, diquafosol within 30 days;

e. Topical cyclosporine, tacrolimus within 6 weeks.

10)Punctal plugs or cautery within 12 weeks.

11)Antiglaucoma drugs within 3 months; non-laser glaucoma surgery; glaucoma laser surgery within 6 months.

12)Nd:YAG capsulotomy within 6 months; corneal refractive surgery (LASIK, etc.) within 12 months.

13)Known allergy to fluorescein, multiple allergies, or severe allergic disease.

14)Uncontrolled severe infection, cardiopulmonary disease, hypertension (SBP ≥150, DBP ≥100), diabetes, malignancy, etc.

15)Positive pregnancy test, lactation; or unwillingness to use contraception during study and 1 month after last dose.

16)Participation in another investigational drug/device study within 30 days.

17)Other conditions deemed ineligible (e.g., depression).

12.6.3 Enrollment Method

Participants will be enrolled in this study in the order they are screened.

12.7 Required Number of Cases

Approximately 20 cases are currently anticipated. This study is an early exploratory study with no definitive statistical hypotheses. The sample size for the treatment group is primarily based on clinical practicality and feasibility, referencing the sample sizes enrolled in published similar clinical studies. For example, in the 2022 clinical study on exosome therapy for dry eye conducted by the LIU laboratory, the treatment group sample size was 14 cases (Sci. Adv. 8, eabj9617 (2022)). Another example is the 2025 clinical study by the Azarpira laboratory on exosome therapy for dry eye, where the treatment group sample size was 8 cases (BMC Ophthalmology (2025) 25:299).

12.8 Method of Use, Dosage, Timing, and Course of Treatment for Stem Cell-Derived Small Extracellular Vesicle Preparation, Including Detailed Procedure if Special Surgical Introduction is Required

12.8.1 Overview of Investigational Drug

12.8.1.1 Method of Obtaining the Investigational Drug

The investigational drug is provided by the Sponsor, uniformly packaged, and has passed quality control testing.

12.8.1.2 Investigational Drug Information

【Generic Name】 Human Mesenchymal Stem Cell Small Extracellular Vesicle Eye Drops (YS245)

【English Name】 Human mesenchymal stem cell-derived extracellular vesicles eye drops

【Pinyin】 ren yuan jian chong zhi gan xi bao xiao xi bao wai nang pao di yan ye

【Active Ingredient】 Human umbilical cord mesenchymal stem cell small extracellular vesicles

【Excipients】 Sterile 0.9% saline solution for injection

【Indication】 This product is indicated for the treatment of signs and symptoms of Dry Eye (DED).

【Specification】 0.65 mL/vial, concentration 3.5×10^9 particles/mL (±20%).

【Dosage and Administration】Instill one drop (approx. 50 μL) into each eye each time, four times daily (morning, noon, evening, night). Approximate weekly usage is 1.4 mL.

【Storage】 Store frozen at -20°C before opening. After opening, store refrigerated at 2-8°C.

【Shelf Life】 One year (-70°C), one week (2-8°C)

【Manufacturer】 Beijing Yisheng Medical Technology Co., Ltd.

12.8.2 Administration

Human mesenchymal stem cell small extracellular vesicle eye drops, one drop per eye each time, four times daily, for 4 weeks.

Treatment Period: Subjects will receive human mesenchymal stem cell small extracellular vesicle eye drops for 4 weeks.

12.8.3 Precautions

This product is for same-day use. Use immediately after opening. After administration, store the remaining solution at 2-8°C.

- Do not use it the next day; it should be returned for disposal.
- Do not touch the tip of the single-dose container to the eye or any surface to avoid eye injury or solution contamination.
- Return unused single-dose containers to their original plastic bag and protect from excessive sunlight exposure.
- Keep out of reach of children.

12.8.4 Missed Dose

Subjects should not compensate for a missed dose. They should continue with the next dose according to the regular schedule. Subject medication compliance will be calculated. Compliance <80% or >120% will be considered a major protocol deviation.

12.8.5 Follow-up Period and Long-term Follow-up Period

After completing the administration in this trial, follow-up visits will occur every 2 weeks for a total of 2 visits. Subsequently, subjects will enter the long-term follow-up period, with follow-up visits at month 3 and month 6 to observe longer-term safety and efficacy.

12.9 Criteria for Discontinuation and Termination of the Clinical Study

Discontinuation of Treatment refers to stopping the investigational drug

treatment and does not imply withdrawal from the study.

Because certain data on clinical events after treatment discontinuation may be very important for the study, this information must be collected up to the subject's last scheduled visit, even if the subject has discontinued treatment.

Subjects may discontinue treatment at any time for any reason, or the investigator may decide to discontinue their treatment if any adverse event occurs. Additionally, the investigator or sponsor may discontinue a subject's treatment if the subject is unsuitable for treatment, violates the study protocol, or for administrative and/or other safety reasons.

If a subject experiences any of the following, the investigator should consider arranging for the subject to discontinue treatment, but the subject may continue to be monitored in the study:

- The subject or their legal representative requests to discontinue treatment;
- Female subject becomes pregnant;
- Occurrence of an adverse event or condition specified in the protocol that requires treatment discontinuation, and continued participation would not be in the subject's best interest;
- The investigator deems it necessary to discontinue treatment (the reason for withdrawal must be documented in detail).

Subject Withdrawal from the Study

Subjects have the right to withdraw from the study at any time for any reason. If a subject withdraws from the study, they will no longer receive treatment or follow-up as per the protocol, but every effort should be made to convince them to complete the treatment discontinuation visit.

The reason for subject withdrawal should be recorded in the electronic Case Report Form (eCRF). Subjects who signed the informed consent form and received study intervention, but withdraw, are withdrawn/terminated from the study, cannot be replaced.

- Subject or their legal representative refuses to participate in further visits;
- Lost to follow-up;
- Death;
- The study is terminated by the Sponsor, Investigator, or Regulatory Authority.
- Early Study Termination/Suspension

Early study termination refers to the premature cessation of all trial activities before the protocol's scheduled end. The purpose of study termination is primarily to protect subject rights, ensure trial quality, and avoid unnecessary economic losses. Before the Sponsor terminates/suspends a clinical trial, they must notify the investigator, the Ethics Committee, and the National Medical Products Administration,

stating the reasons.

Reasons for early study termination/suspension may include:

- Occurrence of serious safety issues during the trial, and the investigator believes subjects' safety may be compromised;
- The investigational drug is found to be too ineffective or even futile during the trial, lacking clinical value;
- A major flaw is found in the clinical trial protocol during the trial, or significant deviations occurred in implementation, making it difficult to evaluate the drug's efficacy and/or safety;
- Available efficacy results support early termination of the study;
- The applicant requests termination (e.g., due to funding issues, management reasons, etc.);
- Request from a higher regulatory authority.

12.10 Efficacy Evaluation Criteria

Primary Efficacy Outcome Measures (at different time points post-administration):

- Total Corneal Fluorescein Staining Score (TCSS) (for subjects positive at baseline);
- Tear Break-Up Time (TBUT).

Secondary Efficacy Outcome Measures:

- OSDI Score;

- Conjunctival Redness Score;
- Schirmer's Tear Test (STT).

12.11 Requirements for Recording Adverse Events, and Methods for Reporting and Handling Serious Adverse Events

Definition of Adverse Events (AEs)

Adverse Event: Any untoward medical occurrence in a subject administered the investigational drug, which may present as symptoms, signs, diseases, or abnormal laboratory findings, but does not necessarily have a causal relationship with the investigational drug.

In this trial, any adverse medical events occurring from the subject's first dose until the last follow-up visit will be collected, categorized as ocular AEs and systemic AEs, collected separately. The end of an adverse event should be recorded when the event resolves, stabilizes without further improvement, is reasonably explained, or the subject is lost to follow-up.

Examples of adverse events include the following situations:

- Worsening of a pre-existing (present before entering the clinical trial) medical condition/disease (including worsening of symptoms, signs, laboratory abnormalities);

- New disease detected or diagnosed after administration of the investigational drug, even if the disease might have existed before the study started;
- Signs, symptoms, or clinical sequelae resulting from suspected overdose of the investigational drug or concomitant medication (the overdose itself is not reported as an AE or SAE).

Definition of Serious Adverse Events (SAEs)

Serious Adverse Event: An adverse medical event occurring in a subject administered the investigational drug 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.

Serious Adverse Event: An adverse medical event occurring at any dose that meets any of the following criteria:

- Results in death;
- Is life-threatening;
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect;

- Other important medical events (may not be immediately life-threatening, result in death, or require hospitalization, but if medical intervention is needed to prevent one of the outcomes listed above, it is generally considered serious. For example, important treatment in an emergency room or allergic bronchospasm occurring at home, cachexia or convulsions not requiring hospitalization, development of drug dependence or addiction.)

Note: "Life-threatening" in the definition refers to a risk of death at the time of the adverse event, not a hypothetical risk of death if the condition were to become more severe in the future.

Handling of Serious Adverse Events: When a serious adverse event occurs, the investigator must immediately take appropriate and active rescue and protective measures for the subject.

- Hospitalization does NOT include the following situations:

- Rehabilitation facilities
- Nursing homes
- Routine emergency room visits

- Same-day surgery (e.g., outpatient/same-day/ambulatory surgery)
- Social reasons (e.g., insurance coverage)
- Hospitalization or prolongation of hospitalization not related to worsening of an adverse event is not itself a serious adverse event. For example:
- Admission for pre-existing disease without a new adverse event or worsening of pre-existing disease (e.g., to check a laboratory abnormality that has persisted since before the trial);
- Hospitalization for administrative reasons (e.g., routine annual physical examination);
- Hospitalization required by the protocol during the clinical trial (e.g., for protocol-mandated procedures);
- Elective hospitalization not related to worsening of an adverse event (e.g., elective cosmetic surgery);
- Pre-planned treatment or surgery should be documented in the protocol and/or the subject's individual baseline data;
- Hospitalization solely for blood product use

12.12 Case Report Form (CRF) See Appendix.

12.13 Statistical Analysis of Study Results

12.13.1 Sample Size Determination

This is an exploratory study with no strict statistical hypotheses. The

sample size was not calculated based on statistical requirements.

12.13.2 Analysis Populations

Describe the definition of the statistical analysis populations. Subjects who did not adhere to the study protocol should be clearly indicated regarding which analysis set they belong to and how missing data will be handled.

12.13.3 Efficacy Analysis and Statistical Methods

For efficacy measures, data for the study eye will be summarized by visit. Results and changes from baseline will be described using descriptive statistics.

12.13.4 Analysis of Primary Outcome Measures

Corneal fluorescein staining scores (ICSS) for the study eye at each follow-up and changes from baseline will be described using descriptive statistics as quantitative variables. Categorical data will be presented as number (percentage) and analyzed using the Chi-square test. $P < 0.05$ indicates a statistically significant difference, $P < 0.01$ indicates a highly statistically significant difference. Tear break-up time (TBUT) at each time point will be described using descriptive statistics, including number, mean, standard deviation, median, minimum, maximum, and 95% confidence interval.

12.13.5 Analysis of Secondary Outcome Measures

Change from baseline to Day 7, Day 14, Day 21, and Day 28:

OSDI Score;

Conjunctival Redness Score;

STT (Schirmer's Tear Test).

Study eye BCVA, BUT, STT, etc., and changes from baseline will be summarized by visit, presenting mean, standard deviation, median, maximum, minimum, upper quartile, and lower quartile. The number and percentage of subjects with study eye BCVA improvement of 5, 10, and 15 letters will be summarized by visit. Appropriate statistical analysis methods will be selected based on data characteristics and general statistical principles.

12.13.6 Safety Analysis and Statistical Methods

Adverse events, laboratory tests, vital signs, 12-lead ECG, safety ophthalmic examination results, and changes from baseline will be described using descriptive statistics. Measurement data will be presented as mean ± standard deviation (x±s). Inter-group comparisons will use two-way repeated measures ANOVA. Intra-group comparisons will use one-way ANOVA. Categorical data will be presented as number (percentage) and analyzed using the Chi-square test. $P < 0.05$ indicates a statistically significant difference, $P < 0.01$ indicates a highly statistically significant difference.

12.13.7 Interim Analysis

To monitor the safety of the clinical trial in a timely manner. If safety issues arise, the trial will be terminated early for safety reasons. The results of the interim analysis will guide subsequent research work and will be statistically analyzed by physicians within the group. Stopping criteria mainly include: foreseeing that continuing the trial to its scheduled end would be unlikely to demonstrate the investigational drug's efficacy, or discovering potential safety concerns with the investigational drug.

12.13.8 Final Analysis

Analyze changes in primary indicators such as corneal fluorescein staining and ocular dryness score before treatment, after 2 weeks of treatment, and after 4 weeks of treatment. Analyze changes in secondary indicators such as visual acuity, intraocular pressure, BUT, and STT. Adverse events, laboratory tests, vital signs, 12-lead ECG, safety ophthalmic examination results, and changes from baseline will be described using descriptive statistics.

12.14 Study Schedule of Assessmen

Assessment Item	V1Screen	V2 D0	V3 D7	V4 D14	V5 D21	V6 D28	V7 D42	V8 D56	V9 D84	V10 D168	EarlyTermination
Informed Consent	✔										
Inclusion/Exclusion Review	✔										
Height / Weight	✔										
Blood Pressure	✔										
Demographics	✔										
Medical History	✔										
Allergy History	✔										
Pregnancy Test	✔						✔		✔	✔	✔

Blood Chemistry	✔	✔					✔		✔	✔	✔
Complete Blood Count	✔	✔					✔		✔	✔	✔
Urinalysis	✔	✔					✔		✔	✔	✔
OSDI	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
BCVA	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Intraocular Pressure	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Slit-Lamp Examination	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Conjunctival Hyperemia Score	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Corneal Fluorescein Staining	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
TBUT	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
STT	✔	✔		✔		✔	✔	✔	✔	✔	✔

Fundoscopy	✔	✔					✔		✔	✔	✔
Eye Drop Comfort Assessment		✔	✔	✔	✔	✔					
IP Dispensing		✔	✔	✔	✔						
IP Retrieval / Adherence			✔	✔	✔	✔					✔
Diary Card		✔	✔	✔	✔	✔					✔
Concomitant Medications	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Adverse Events	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔

Notes

- Screening period: D-14 to D-1. All screening procedures must be completed prior to the first dose. Treatment period: 28 days. Safety follow-up period: 28 days. For successfully enrolled subjects, results obtained within 7 days before Visit 2 (V2) may be used to waive duplicate tests

- Medical history and treatment: Document dry eye history, all prior treatment and surgery history, significant past medical history, and all medical conditions within 60 days prior to screening.
- Pregnancy test: For women of childbearing potential, serum pregnancy test is preferred; urine pregnancy test is also acceptable. Tests will be performed at screening, end of treatment, or early termination.
- Blood biochemistry: Glucose, total bilirubin, direct bilirubin, cholesterol, triglyceride, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, calcium, sodium, potassium, chloride, urea/urea nitrogen, creatinine, uric acid. Results within 7 days before V2 may be used to waive duplicate tests.
- Complete blood count: White blood cell count, absolute neutrophil count, absolute eosinophil count, absolute basophil count, absolute lymphocyte count, red blood cell count, hemoglobin, platelet count. Results within 7 days before V2 may be used to waive duplicate tests.
- Urinalysis: Urine protein, urine red blood cells, urine white blood cells, urine glucose, urine ketones, urine specific gravity, urine pH, urobilinogen. Results within 7 days before V2 may be used to waive duplicate tests.

- BCVA (Best-corrected visual acuity): Must be assessed before instillation of artificial tears or investigational product at all relevant visits.
- Slit-lamp microscopy: Must be performed before instillation of artificial tears or investigational product at all relevant visits.
- BUT (Tear film break-up time): Must be completed at least 15 minutes prior to STT.
- Fundoscopy: To be performed at the end of each relevant visit, after all other ocular examinations and assessments, and before investigational product administration.
- Eye drop comfort assessment: To be completed by the subject with the assistance of trained study personnel immediately after investigational product instillation at the study site, at approximately 3 minutes. If the score is >3 at 3 minutes, repeat assessments will be performed at approximately 5, 10, and 15 minutes until the score is ≤3. If the score remains >3 at 15 minutes, this will be documented as an adverse event (AE).

- Collect returned artificial tears and investigational product, assess medication compliance, and retrieve all empty containers before dispensing new study medication. Instruct subjects not to self-administer the study drug on the day of the on-site visit prior to the visit.
- At V2, V3, V4, V5, and V6 visits, trained staff will provide investigational product administration instructions approximately 15 minutes after completion of all study assessments except eye drop comfort assessment. Subjects will be instructed not to use the study drug for 2 hours before the on-site visit examinations on the day of the visit. At V7 and V8 visits, safety and efficacy assessments will be performed. At V9, V10, and early termination, long-term safety and efficacy will be evaluated.
- Concomitant medications: Document all concomitant ocular and systemic medications during screening and treatment periods. After the last dose, only concomitant medications related to investigational product-associated AEs will be recorded.

- Adverse events (AEs): All AEs will be collected from the first dose until the final visit, classified and documented separately as ocular AEs and systemic AEs. All AEs will be followed until resolution, stabilization with no further improvement, satisfactory explanation, or subject loss to follow-up.

12.15 Major Risks and Risk Mitigation Plan

12.15.1 Major Risks

As an emerging biological therapy, the risks of human mesenchymal stem cell small extracellular vesicle eye drops primarily stem from the product's inherent characteristics, manufacturing process, and immune responses.

1) Immunogenicity and Inflammatory Reaction Risk

Risk Description: Although human MSC-EVs are considered low immunogenicity, allogeneic (e.g., umbilical cord, placental stem cell) derived human MSC-EVs may still carry donor-specific antigens or bioactive substances that could potentially induce local ocular surface immune-inflammatory reactions, such as conjunctival redness, worsening foreign body sensation, or immune cell infiltration.

Scientific Mechanism: MHC class I molecules on the surface of human MSC-EVs or certain proteins within their contents may be recognized by the host immune system, activating T cells or triggering complement reactions.

2) Product-Related Impurity and Contamination Risk

Risk Description: During the preparation process, human MSC-EVs may co-isolate apoptotic bodies, microvesicles, protein aggregates, or cellular debris as impurities. Additionally, there is a risk of microbial contamination (bacteria, endotoxins, mycoplasma), which could cause infection or severe inflammation if directly instilled into the eye.

Core Challenge: The extraction and purification processes for human MSC-EVs (e.g., ultracentrifugation, size exclusion chromatography, PEG precipitation) have not yet formed a unified "gold standard." The purity, concentration, and vesicle integrity of different batches may vary, directly impacting safety.

3) Efficacy Uncertainty and Disease Modification Risk

Risk Description: Human MSC-EVs exert their effects by modulating immunity and promoting repair. However, if improperly regulated, they could theoretically disrupt local immune balance, suppressing necessary immune defense functions, or lead to abnormal tissue repair (e.g., fibrosis).

Dose Dependency: Too low a dose may be ineffective, while too high a dose might exacerbate inflammation or produce other unknown side effects. The therapeutic window requires precise exploration.

12.15.2 Risk Mitigation Plan

To address the above risks, multi-level, proactive risk control strategies

need to be developed during R&D and clinical application.

Risk Type	**Risk Mitigation Plan and Strategy**
Immunogenicity Risk	- Source Selection: Prioritize allogeneic umbilical cord-derived human MSCs, known for lower immunogenicity. Their derived small EVs possess stronger and safer immunomodulatory capabilities. - Strict Quality Control: Identify specific small EV markers (e.g., CD9, CD63, CD81) in the final product and detect HLA molecule expression levels. - Clinical Monitoring: Monitor changes in ocular surface inflammatory indicators (e.g., conjunctival redness score, patient-reported ocular discomfort) during the clinical trial as appropriate.
Impurity & Contamination Risk	- Process Development: Establish and standardize high-purity, high-yield production processes based on tangential flow filtration, size exclusion chromatography, etc., ensuring batch-to-batch consistency. - Release Testing: Establish strict release specifications, including:

Risk Type	Risk Mitigation Plan and Strategy
	① Sterility and Endotoxin Testing (must comply with pharmacopoeia standards for ophthalmic preparations). ② Multi-dimensional quality control: Electron microscopy (morphology), NTA (particle size distribution and concentration), WB (marker protein validation). ③ Impurity Residue Testing: Measure residual amounts of non-exosome components such as proteins and nucleic acids.
Efficacy & Regulation Risk	- Dose Exploration: Design a rigorous Phase I clinical trial with multiple dose-escalation cohorts to find the optimal safe and effective dose. - Patient Selection: Define clear inclusion/exclusion criteria in the clinical trial, e.g., exclude patients with active ocular infection, history of ocular tumors, or active phase of severe autoimmune diseases.

Risk Type	Risk Mitigation Plan and Strategy
	- Discontinuation Criteria: Establish clear stopping rules for pausing or terminating administration, such as the occurrence of intolerable inflammatory reactions or vision decline