A Single-Center Clinical Study Evaluating the Efficacy and Safety of Allogeneic Natural Killer (NK) Cell Transplantation for Mooren's Ulcer

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A Single-Center Clinical Study Evaluating the Efficacy and Safety of Allogeneic Natural Killer (NK) Cell Transplantation for Mooren's Ulcer

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Protocol Summary

Study Title: A Single-Center Clinical Study Evaluating the Efficacy and Safety of Allogeneic Natural Killer (NK) Cell Transplantation for Mooren's Ulcer.

Study Objective: This study enrolls patients with Mooren's ulcer to exploratively investigate the safety and efficacy (therapeutic outcomes) of allogeneic natural killer (NK) cell transplantation therapy.

Study Subjects: Patients with Mooren's ulcer, especially those with multiple treatment failures.

Study Design: An investigator-initiated, single-arm, prospective Phase Ib dose-escalation clinical trial.

Planned Number of Subjects:

A total of 6 cases.

Study Period and Expected Participant Involvement Duration:

Study Period: May 2026 – November 2027

Expected Participant Involvement Duration:

The expected participation period for each subject in this clinical study is from the date of informed consent and treatment to the end of the observation period (48 weeks after NK cell transplantation).

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1. Background Information

Mooren’s ulcer is an idiopathic, progressive and painful corneal ulcer, representing a unique autoimmune corneal disease confined to the cornea. Lesions initially emerge at the corneal periphery, extend bilaterally along the limbus, then progress centripetally in an invasive manner, and may eventually involve the entire cornea. The incidence is slightly higher in males than females, with 70% of patients presenting with unilateral disease. Based on clinical manifestations and prognosis, Mooren’s ulcer is classified into benign and malignant subtypes. The benign type typically manifests unilaterally and predominantly affects elderly patients; the malignant type is frequently bilateral, characterized by severe, rapidly progressive disease, and occurs mostly in younger individuals.

Mechanistically, Mooren’s ulcer is categorized as an autoimmune disorder, yet its exact pathological mechanism remains incompletely elucidated. Studies have demonstrated deposition of immunoglobulins and complement C3 in conjunctival tissue from ulcerative lesions; circulating autoantibodies targeting corneal and conjunctival epithelia can be detected in patient serum. Meanwhile, patients exhibit reduced numbers of peripheral blood suppressor T cells alongside an elevated helper T cell/suppressor T cell ratio. Previous proteomic and single-cell RNA sequencing analyses have revealed prominent infiltration and functional dysregulation of T cells, B lymphocytes and natural killer (NK) cells within ulcer lesions, accompanied by markedly elevated immunoglobulin-related components in diseased tissue. Additionally, significantly increased components associated with neutrophil extracellular traps (NETs) have been identified in lesional tissues. Current mainstream therapeutic strategies for Mooren’s ulcer are as follows: pharmacotherapy serves as the first-line treatment for mild cases, while combined surgical and medical intervention is adopted for severe patients or those with unsatisfactory drug responses. Although approximately 90% of patients achieve disease remission, recurrence is common, and no curative therapy is available to date.

Natural killer (NK) cells are core components of the innate immune system, whose activity is primarily regulated by balanced signaling

between activating and inhibitory receptors. NK cells exert immune surveillance and maintain tissue homeostasis mainly through perforin/granzyme secretion, death receptor-mediated apoptosis, and antibody-dependent cellular cytotoxicity (ADCC). Compared with CAR-T therapy, NK cell transplantation exhibits superior safety profiles for the following reasons: activated NK cells predominantly secrete IFN-γ, whereas CAR-T cells induce the production of multiple cytokines linked to cytokine release syndrome (CRS), including IL-1, IL-2, TNF and IL-6. Moreover, NK cells lack T cell receptors and generally do not trigger graft-versus-host disease (GVHD). Accumulating studies have demonstrated the protective effects of NK cells in autoimmune diseases such as systemic lupus erythematosus (SLE), and adoptive transfer of NK cells can delay disease onset of autoimmune disorders. A research team from Changhai Hospital, Naval Medical University has confirmed in clinical trials that CD19 CAR-NK cells can effectively treat refractory systemic lupus erythematosus, highlighting the prominent advantages of NK cell-based therapy for autoimmune diseases.

Our previous single-cell sequencing results revealed that NK cells in lesional tissues of Mooren’s ulcer are abnormally activated, suggesting their potential involvement in disease pathogenesis. Given that NK cells lack T cell receptors and possess a unique self/non-self recognition mechanism, transplantation of NK cells generally does not induce

graft-versus-host disease (GVHD), rendering them an ideal vehicle for cell therapy. In our prior rabbit model experiments, we verified the safety of NK cell transplantation. Compared with the control group, conjunctival NK cell administration showed no significant toxic effects on corneal epithelial integrity, corneal endothelial cell morphology, or conjunctival tissues. Considering the protective effects of NK cells in autoimmune diseases including systemic lupus erythematosus, this project aims to investigate the efficacy and safety of allogeneic NK cell transplantation for Mooren's ulcer, so as to provide a novel therapeutic option for refractory Mooren's ulcer.

2. Objectives

This clinical study primarily enrolls patients with refractory Mooren's ulcer who failed conventional therapy (persistent corneal thinning) or experienced disease recurrence. The primary objective is to evaluate the safety of allogeneic NK cell transplantation, while the secondary objective is to assess the therapeutic efficacy of this transplantation regimen, so as to explore the feasibility of allogeneic NK cell transplantation for the treatment of refractory Mooren's ulcer.

2.1 Primary Objective

The primary objective of this clinical study is to evaluate the safety of allogeneic NK cell transplantation for the treatment of Mooren's ulcer. Based on indicators from clinical physical examinations and auxiliary

tests, safety will be assessed by analyzing the incidence, severity and frequency of ocular and systemic adverse events. In addition, ocular safety will be evaluated according to the severity and frequency of the following adverse outcomes: survival of non-target cells, elevated intraocular pressure, rejection reaction, infection, cataract, glaucoma, progression of retinal and fundus lesions, and expulsive hemorrhage.

2.2 Secondary Objective

The secondary objective of this clinical study is to evaluate the therapeutic efficacy of allogeneic NK cell transplantation. Efficacy will be assessed based on slit-lamp biomicroscopy-derived indicators including inflammatory severity, corneal epithelial defect, corneal thinning, and best corrected visual acuity (BCVA).

3. Study Design and Evaluation Items

3.1 Study Type

Investigator-initiated exploratory clinical study.

3.2 Study Design

Single-arm, prospective Phase Ib dose-escalation trial.

3.3 Evaluation Items

3.3.1 Primary Endpoints: Safety Endpoints

1. Systemic safety endpoints: Classification of the severity of toxic adverse reactions following allogeneic NK cell transplantation; assessment of the type, severity and incidence of all related adverse

events.

2. Ocular safety endpoints: Proliferation and survival of non-target cells, elevated intraocular pressure, rejection reaction, infection, cataract, glaucoma, progression of retinal and fundus lesions, expulsive hemorrhage, corneal thinning and perforation.

3.3.2 Secondary Endpoints (Efficacy Endpoints):

Inflammatory severity, corneal epithelial defect, corneal thinning status assessed via slit-lamp biomicroscopy, and best corrected visual acuity (BCVA).

3.4 Study Method Overview

This is a single-center, single-arm, prospective Phase Ib dose-escalation clinical trial enrolling patients with Mooren’s ulcer to evaluate the safety and efficacy of allogeneic natural killer (NK) cell transplantation.

The study consists of three phases: screening period, transplantation period, and observation period.

After obtaining written informed consent and completing study registration, patients will undergo screening examinations to verify that they meet all inclusion criteria and do not satisfy any exclusion criteria. On the scheduled transplantation day (Day 0), allogeneic NK cell transplantation will be performed on each subject. NK cells at different doses will be injected into the conjunctival fornix using a 30-gauge

needle: low-dose group (1×10^6 cells, 500 μL), medium-dose group (3×10^6 cells, 500 μL), and high-dose group (6×10^6 cells, 500 μL). Subjects will be admitted to hospital starting two days before transplantation (Day -2).

On Day (5±2) post-transplantation, slit-lamp biomicroscopy, tonometry, ocular optical coherence tomography (OCT), and ocular B-scan ultrasonography will be performed to examine for significant elevated intraocular pressure, rejection, expulsive hemorrhage, progression of cataract or glaucoma, corneal thinning, corneal perforation, and other adverse findings. If any of the above conditions occur, the principal investigator and attending physicians will provide timely clinical management. Subjects may be discharged only after clinical judgment confirms no further inpatient monitoring is required.

During the 48-week observation period following allogeneic NK cell transplantation, subjects are required to complete a total of 8 follow-up visits: Visit 2 (Week 1), Visit 3 (Week 2), Visit 4 (Week 4), Visit 5 (Week 8), Visit 6 (Week 12), Visit 7 (Week 24), Visit 8 (Week 32), and Visit 9 (Week 48). If any safety concerns arise from study findings, necessary additional interventions shall be implemented, followed by a supplementary report submitted to the Ethics Committee for re-approval.

The Ethics Committee is responsible for the overall safety oversight of this clinical trial. After transplantation of the first subject, the trial principal investigator may request an Ethics Committee meeting based on

the occurrence of serious adverse events or other relevant clinical findings to seek committee opinions regarding study continuation or amendments to the clinical trial protocol.

3.5 Expected Participation Duration of Subjects

The anticipated participation period for each subject in this clinical study spans from the date of signing the informed consent form (1 week prior to allogeneic NK cell transplantation) to the end of the observation period (48 weeks after NK cell transplantation).

4. Subject Inclusion, Exclusion and Discontinuation Criteria

4.1 Inclusion Criteria

Patients satisfying all the following criteria are eligible for enrollment:

1. Clinically diagnosed with Mooren’s ulcer consistent with its clinical diagnostic criteria: peripheral corneal infiltration and ulcer with progressive progression accompanied by ciliary congestion, with other corneal disorders excluded.
2. Ulcerative lesion extent ≤ 1/2 of corneal circumference; stromal infiltration depth ranging from 1/3 to 2/3 of full corneal thickness; no severe complications such as corneal perforation or endophthalmitis.
3. Present with obvious ocular symptoms including ocular pain, photophobia, lacrimation and foreign body sensation, with poor

response to conventional conservative pharmacotherapy.

4. Intraocular pressure of the affected eye within normal range (10–21 mmHg); no concurrent severe blepharitis, dacryocystitis or other ocular diseases; no history or active lesions of Mooren’s ulcer in the contralateral eye.
5. Aged between 18 and 85 years at the time of consent, regardless of gender.
6. Capable of providing written informed consent to voluntarily participate in this study.

4.2 Exclusion Criteria

Patients meeting any of the following conditions shall be excluded:

1. Failure to meet any single item of the above inclusion criteria.
2. Corneal lesion-related exclusions: severe complications including corneal perforation, endophthalmitis, corneal staphyloma; ulcer extent > 1/2 corneal circumference or stromal infiltration depth > 2/3 corneal thickness; concurrent other corneal diseases (e.g. infectious keratitis, keratoconus, corneal degeneration).
3. Systemic disease-related exclusions: uncontrolled severe cardiovascular and cerebrovascular diseases, hepatic or renal insufficiency (ALT/AST > 1.5 times upper limit of normal (ULN), serum creatinine > 1.2 times ULN), hematopoietic dysfunction, uncontrolled autoimmune diseases (e.g. rheumatoid arthritis,

systemic lupus erythematosus); active infection, malignant tumors, or surgical history within the past 3 months; poorly controlled diabetes with HbA1C ≥ 8.5%.

4. Special population exclusions: pregnant or breastfeeding women; subjects of both genders with pregnancy plans without effective contraception; alcohol dependence or drug abuse history.
5. Other exclusions: inability to cooperate with trial follow-up and examinations; other factors interfering with trial outcome assessment (e.g. history of ocular trauma, congenital ocular malformations); subjects judged ineligible for enrollment by investigators.
6. Participation in another clinical trial within 3 months prior to signing informed consent.
7. Subjects deemed unsuitable for this clinical trial due to concomitant comorbidities or other conditions.

4.3 Subject Discontinuation Criteria

Subject participation in this clinical trial shall be terminated immediately if any of the following situations occur during the trial, with complete follow-up and safety records maintained, and corresponding medical interventions administered when necessary.

1. Subject voluntary withdrawal due to personal reasons (e.g., intolerance to trial treatment). Participation may be terminated after

the subject signs the discontinuation informed consent form.

2. Occurrence of serious adverse events: Severe adverse reactions emerge during the trial, including severe allergic reactions, aggravated corneal perforation, endophthalmitis, severe systemic infection, or other life-threatening conditions. If investigators determine continued trial participation poses safety risks to the subject, participation shall be terminated without delay.
3. Disease progression or treatment failure: The Mooren's ulcer continuously deteriorates during the trial (expanded ulcer area, deeper stromal infiltration, signs of perforation, etc.), or symptoms show no improvement or even worsen after 4 weeks of treatment. Investigators will discontinue trial treatment and switch the subject to alternative effective therapeutic regimens if the study treatment is deemed ineffective.
4. New concurrent diseases: New severe systemic or ocular diseases develop during the trial that may interfere with trial outcomes or endanger the subject's safety, as judged by investigators.
5. Loss to follow-up: The subject cannot be contacted, or refuses to attend scheduled follow-up visits and examinations, resulting in incomplete trial data. Their participation will be terminated.
6. Overall suspension of this clinical trial.

4.4 Management After Subject Discontinuation

After a subject discontinues trial participation, investigators shall complete the final follow-up examination and document the reason and date of discontinuation. All adverse events shall be followed up until symptoms are relieved or stabilized. All trial data of the subject shall be properly preserved to guarantee integrity, authenticity and traceability. Investigators shall inform the subject of post-discontinuation precautions and recommendations for subsequent treatment.

5. Obtaining Subject Consent and Study Registration

The principal investigator or attending physician shall verbally and in writing explain the trial purpose, procedures and other relevant information to the subject, and obtain written informed consent for study participation.

5.1 Development of Informed Consent Form and Subject Information Sheet

The principal investigator shall draft the informed consent form and subject information sheet, which must be approved by the Ethics Committee prior to use.

5.2 Timing and Procedures for Consent Acquisition

When seeking consent from the patient, the principal investigator or attending physician shall provide the subject information sheet and

explain the study purpose, procedures and relevant details in plain language. Subsequently, all questions raised by the patient regarding the study shall be answered to confirm full understanding of study contents. Finally, the investigator shall verify that the patient voluntarily agrees to participate in this clinical trial based on independent judgment, which will be documented by the patient’s dated signature or seal on the informed consent form.

During consent acquisition, the principal investigator or attending physician shall record the patient’s contact information and the contact details of emergency contacts such as family members in medical records under institutional management.

5.3 Acquisition, Storage of Informed Consent Forms and Subject Registration

The principal investigator or attending physician who provides study explanations and the subject shall each date and sign or affix a seal on the informed consent form. The original copy shall be retained by the principal investigator or attending physician. The copy shall be processed with linkable anonymization by the personal information manager for storage. After consent is obtained, the principal investigator or attending physician shall submit the subject’s information to the clinical registry for enrollment registration. A dated signed copy of the informed consent form shall be provided to the subject before study participation, and the

delivery date shall be documented.

5.4 Documentation of Consent Date

The principal investigator or physician in charge of regenerative therapy shall record the date of informed consent acquisition from the subject in the case report form (CRF).

6. Study Implementation Schedule

6.1 Screening Period: 1 Week Prior to Transplantation

6.1.1 Consent Acquisition

Prior to any trial-related examinations or observations, the principal investigator or attending physician shall obtain written informed consent from the patient and assign a unique subject identification number. Consent acquisition shall be completed within 1 week before transplantation. In addition, the subject’s willingness to participate will be reconfirmed at the first screening examination.

6.1.2 Eligibility Verification

The principal investigator and attending physician shall conduct preliminary eligibility verification via designated examinations and assessments to confirm whether candidate subjects meet all inclusion criteria and none of the exclusion criteria. Final eligibility confirmation will be performed 2 to 3 days before transplantation based on all screening test results. Both preliminary and final eligibility verifications shall be carried out in accordance with the inclusion criteria in Section

4.1 and exclusion criteria in Section 4.2.

6.1.3 Day Prior to Transplantation (Day -1)

The day before allogeneic NK cell transplantation for subjects is defined as Day -1.

(1) Trial-related ophthalmic examinations for subjects: slit-lamp biomicroscopy (anterior segment), intraocular pressure measurement, assessment of conjunctival congestion, corneal confocal microscopy, B-scan ultrasonography, anterior segment optical coherence tomography (AS-OCT), fundus photography, and visual acuity tests (uncorrected and best corrected visual acuity).

(2) Concomitant therapies and concomitant medications.

6.1.4 Day of Transplantation (Day 0)

(1) Subject body temperature and blood pressure will be measured in the morning on Day 0. The transplantation procedure shall be suspended or postponed if the subject’s body temperature ≥ 38°C, or severe hypertension is present (systolic blood pressure > 200 mmHg, diastolic blood pressure > 120 mmHg).

(2) Subjects will receive ophthalmic examinations including slit-lamp biomicroscopy with anterior segment photography and intraocular pressure measurement, as well as evaluation of conjunctival congestion and other ocular conditions to confirm the feasibility of NK cell transplantation.

6.1.5 Days 1 to 5 Post-Transplantation

(1) Subjects will undergo ophthalmic examinations: visual acuity test, slit-lamp biomicroscopy (anterior segment), intraocular pressure measurement, and evaluation with grading of conjunctival congestion.

6.1.6 Day (7±2) Post-Transplantation

(1) Subjects will receive ophthalmic examinations including visual acuity test, slit-lamp biomicroscopy (anterior segment), intraocular pressure measurement, graded conjunctival congestion assessment, anterior segment OCT, fundus examination and B-scan ultrasonography.

6.1.7 Day of Discharge

(1) Subjects will receive ophthalmic examinations: visual acuity test, slit-lamp biomicroscopy (anterior segment), intraocular pressure measurement, and graded conjunctival congestion assessment.

(2) Investigators shall document the subject’s ocular status and any adverse events in detail within the electronic medical records.

6.2 Observation Period: Visits 3 to 9 (Week 2 to Week 48)

The scheduled follow-up visits include Visit 3 (Week 2), Visit 4 (Week 4), Visit 5 (Week 8), Visit 6 (Week 12), Visit 7 (Week 24), Visit 8 (Week 32), and Visit 9 (Week 48). All required examinations and assessments shall be completed within the corresponding visit windows following the procedures below:

1. Investigators will inquire about the subject’s symptoms, signs and

any adverse events occurring since the last visit, and record all details in the electronic medical records.

2. Subjects will complete ophthalmic examinations: visual acuity testing, slit-lamp biomicroscopy with anterior segment photography, corneal confocal microscopy or specular microscopy, intraocular pressure measurement, B-scan ultrasonography (when clinically indicated), and anterior segment OCT.

6.3 Unscheduled Visits

At any stage of the trial, if subjects develop uncomfortable symptoms or signs outside scheduled examination time points, unscheduled visits shall be arranged as clinically necessary, with corresponding examinations performed and all findings documented in a timely manner.

7. Examinations and Assessments

7.1 Subject Baseline Information

Baseline items include subject gender, date of birth, date of informed consent acquisition, target disease (diagnosis name, study eye), disease treatment history (history of prior treatment, treatment duration and modalities, history of transplantation, transplantation timing and surgical procedures, etc.), current medical history, concomitant diseases, past medical history, history of drug allergy, concomitant medications, and pregnancy status.

7.2 Baseline Examinations

7.2.1 Visual Acuity Examination

Measure 5-meter visual acuity (uncorrected and corrected) of the study eye receiving transplantation.

7.2.2 Intraocular Pressure Measurement

Intraocular pressure of the study eye will be measured with a handheld tonometer or air-puff non-contact tonometer.

7.2.3 Slit-Lamp Corneal Examination

Slit-lamp biomicroscopy is used to assess corneal opacification and inflammatory severity, corneal epithelial defect and its relative area, corneal stromal thinning, and presence of corneal perforation.

7.2.4 Corneal Confocal Microscopy and OCT Examinations

Anterior segment OCT of the study eye is performed to detect changes in central and peripheral corneal thickness. Confocal microscopy or corneal specular microscopy is applied to observe morphological and density changes of corneal endothelial cells in the study eye, as well as the infiltration and distribution of immune cells in corneal tissue.

7.3 Conjunctival Ophthalmic Examinations

Slit-lamp biomicroscopy will be performed to evaluate the following conjunctival indicators of the study eye.

Item	Grading

Conjunctival hyperemi	0.None 1.Mild 2.Moderate 3.Severe 9.Uncheckable
Ciliary hyperemia	0.None 1.Mild 2.Moderate 3.Severe 9. Uncheckable
Ocular discharge	0.None 1.Scanty 2.Moderate 3.Profuse 9.Uncheckable

7.4 Other Assessments

Document the clinical manifestations, onset time, and corresponding interventions of rejection reactions occurring in the study eye after NK cell transplantation.

7.5 Clinical Laboratory Tests

7.5.1 Hematological Tests

Laboratory examination items

Examination Item	Examination Indicators
Routine blood test	White Blood Cell count (WBC), Red Blood Cell count (RBC), Platelet count (PLT), neutrophil %, lymphocyte %, monocyte %, hemoglobin (HGB)
Urine routine	Urine occult blood, Urine pH, RBC count, WBC count, Urine glucose, Urine protein, Urine ketone bodies, Urine specific gravity
	Liver function: Total bilirubin (TBIL), Direct bilirubin (DBIL), Indirect bilirubin (IBIL), Aspartate aminotransferase

Blood biochemistry	(AST), Alanine aminotransferase (ALT), Gamma-glutamyl transferase (GGT), Alkaline phosphatase (ALP), Total protein (TP), Albumin (ALB); Renal function: Blood urea nitrogen (BUN), Creatinine (Cr); Electrolytes: Calcium (Ca^{2+}), chloride (Cl^{-}), potassium (K^{+}), sodium (Na^{+}), magnesium (Mg^{2+}); Myocardial enzyme profile: Creatine kinase (CK), CK-MB, Lactate dehydrogenase (LDH)
Blood glucose	Fasting blood glucose, Glycated hemoglobin (HbA1c)
Autoimmune antibodies	Rheumatoid factor (RF), antinuclear antibody (ANA), anti-double-stranded DNA antibody (anti-dsDNA), anti-cyclic citrullinated peptide antibody (anti-CCP), anti-neutrophil cytoplasmic antibody (ANCA)
Coagulation function (four items)	Activated Partial Thromboplastin Time (APTT), International Normalized Ratio (INR), Prothrombin activity (PT%), Prothrombin Time Ratio (PT-R), Fibrinogen (FIB), Prothrombin Time (PT), Thrombin Time (TT)
	Hepatitis B core antibody (HBcAb),

Viral screening (four items)	Hepatitis B e antigen (HBeAg), Hepatitis B surface antigen (HBsAg), Hepatitis B e antibody (HBeAb), Hepatitis B surface antibody (HBsAb), HIV (1+2) antibody (HIV-Ab), Hepatitis C antibody (HCV-Ab), Treponema pallidum antibody (TP-Ab)

7.5.2 Other Preoperative Ophthalmic Laboratory Tests

Test items: electrocardiogram (ECG), serological infection screening (syphilis, HBV, HCV), routine blood test, autoimmune antibody panel.

7.6 Adverse Events

Adverse events will be identified through clinical examinations during hospitalization and outpatient visits.

8. Specially Processed Cellular Product

8.1 Overview of Specially Processed Cellular Product

Product Name: Clinical-grade Peripheral Blood-derived NK Cells (NK010), Shanghai NK Therapeutics

Component	Volume/Amount
Allogeneic natural killer cells	$1\text{-}6\times10^{6}$ cells

8.2 Pre-transplant Preparation

Prior to subconjunctival transplantation, the aliquoted NK-cell suspension cryopreserved in liquid-nitrogen tanks is rapidly thawed in a

37- water-bath. After thawing, the cryovial is wiped with ethanol swabs and the sealing film is removed. A 1-mL syringe is inserted through the cap of the cryovial to draw 500 µL of the thawed cell suspension, followed by immediate replacement with a 30-gauge needle.

8.3 Allogeneic Natural Killer Cell Administration

On the day of transplantation, the decision to proceed with allogeneic NK cell transplantation shall be made by the principal investigator together with a minimum of two conflict free physicians. Their comprehensive assessment includes the subject's real time condition and the status of cells from their release until initiation of the procedure.

Before allogeneic NK-cell transplantation, 0.5% proparacaine hydrochloride eye-drops are instilled onto the ocular surface. The eyelid is disinfected with cotton-tipped applicators soaked in 5% povidone-iodine, followed by topical administration of 0.3% gatifloxacin to the treated eye. A sterile eyelid speculum is placed. Cotton swabs moistened with 0.5% proparacaine hydrochloride are used to manipulate conjunctiva near the limbus to confirm its mobility. Alternative injection sites are selected if conjunctival scarring is present. The operator must wear sterile gloves during subconjunctival injection. The injection quadrant is chosen as the one closest to Mooren's ulcer. The low-dose group receives a single injection whereas the highest-dose group

undergoes two separate injections in the two quadrants adjacent to the corneal ulcer. Patients are instructed to gaze away from the injection site. A 30-gauge needle is advanced subconjunctivally with blunt dissection. The predetermined volume of cell suspension is delivered into the subconjunctival space near the limbus; successful placement is verified by visible conjunctival elevation after injection. After transplantation, 0.3% gatifloxacin is administered topically, and a bandage contact lens is fitted depending on ocular-surface conditions. The dose stratification is shown below: low-dose group (1×10^6 cells in 500 μL), intermediate-dose group (3×10^6 cells in 500 μL), high-dose group (6×10^6 cells in 500 μL).

Detailed information of allogeneic NK cell transplantation, including the transplantation date, anesthesia method, presence or absence of combined surgery, concomitant treatments, and operation duration, as well as the occurrence of intraoperative complications, shall be fully documented in the medical records.

8.4 Management of Insufficient Therapeutic Efficacy

At Week 24, an efficacy evaluation shall be conducted by the Ethics Committee. If the transplanted cells are assessed as "failing to exert sufficient therapeutic effect" in the efficacy judgment items, the investigator shall confirm the subject’s willingness to receive re-transplantation surgery via interview after Week 24 and prior to the completion of follow-up for the clinical study. If the subject wishes to

undergo re-transplantation surgery and the attending physician determines that the benefits of re-transplantation outweigh its risks and harms, one re-operation (with unrestricted surgical procedure) may be performed using study funds, limited to a single occurrence only.

8.5 Safety-Related Measures to Ensure the Safety of Investigational Treatment

8.5.1 Allogeneic Natural Killer Cells

8.5.2 Medium Supplements for Isolation and Culture

Proteins and cytokines contained in the ITS supplement added to the medium are all human recombinant proteins. No animal-derived or human-derived products are included.

8.5.3 Safety Tests for Culture Medium and Preservation Solution of Allogeneic Natural Killer Cells

Samples of culture medium or preservation solution shall be taken during cell culture and pre-release preservation throughout manufacturing processes for sterility test and endotoxin testing. Transplantation shall be terminated if the testing result during preparation or preservation is positive (endotoxin level 0.25 EU/mL).

8.6 Benefits and Risks of Allogeneic Natural Killer-Cell Transplantation

8.6.1 Anticipated Benefits

Allogeneic natural killer (NK)-cell transplantation is expected to

markedly relieve clinical manifestations of Mooren’s ulcer and effectively improve patients’ visual acuity.

8.6.2 Anticipated Risks

(1) Adverse events induced by allogeneic natural killer-cell transplantation

Following allogeneic NK-cell transplantation, possible adverse outcomes include elevated intraocular pressure, graft-versus-host disease, anterior-chamber inflammation, corneal endothelial cell injury and proliferation of target cells, which may further lead to decreased visual acuity. The notable symptoms, signs and corresponding management measures are listed below.

Elevated Intraocular Pressure

Judgment Criteria: Intraocular pressure (IOP) ≥25 mmHg recorded during the observation period.

Management Measures: The principal investigator and attending physicians shall discuss the therapeutic regimen within two weeks and arrange follow-up visits for subjects. Intraocular-pressure-lowering medications will be initiated, including one or multiple agents from prostaglandin analogues, beta-blockers, carbonic anhydrase inhibitors and ROCK inhibitors. Subjects are required to return for re-evaluation within 4 weeks after treatment. If the IOP drops below 20 mmHg, the principal investigator and attending physicians will decide whether to continue

hypotensive medication. If intraocular pressure fails to decline, the principal investigator and attending physicians will consult glaucoma specialists to formulate an appropriate treatment plan.

Graft-versus-Host Disease (Immune-Mediated Rejection)

Judgment Criteria: Increased keratic precipitates (KPs) and worsening corneal edema. However, if corneal thickness improves after treatment with topical steroid eye drops, the condition shall be judged as suspected rejection.

Management Measures: Within 1 week after confirmation of increased KPs and aggravated corneal edema consistent with the above criteria, the principal investigator and study physicians shall discuss the therapeutic regimen and arrange follow-up for subjects. While monitoring subjects' clinical findings, intensified topical steroid eye drops (administered every 1-2 hours) and systemic intravenous steroid infusion shall be added. (Example): Intravenous infusion of methylprednisolone once daily for 3-5 consecutive days.

Anterior Chamber Inflammation and Corneal Endothelial Cell Loss

Judgment Criteria: Inflammatory cells and protein flare within the aqueous humor are detected by ocular OCT or slit-lamp biomicroscopy. Irregular cell morphology and reduced cell density are identified via specular microscopy.

Management Measures: The principal investigator and study

physicians shall discuss the treatment regimen and arrange follow-up visits for subjects. For mild endothelial injury, topical glucocorticoid eye drops (e.g., 1% prednisolone acetate) combined with growth-factor eye drops are prescribed, and the dosage is tapered gradually based on inflammation subsidence. For moderate injury, high-frequency topical steroids are administered with ROCK inhibitors added when necessary. In cases of severe injury, systemic medication (oral or intravenous route) is adopted. The risk of corneal endothelial decompensation will be evaluated, and corneal transplantation will be performed if indicated.

Proliferation of Target Cells

Judgment Criteria: Mass-like cell aggregates or tumor-like lesions in the anterior chamber and posterior segment (vitreous cavity and retina) are identified on ocular OCT or slit-lamp biomicroscopy examination.

Management Measures: After maintaining intraocular space with ophthalmic viscoelastic substances, cell masses shall be removed as thoroughly as possible using intra-anterior-chamber forceps. Appropriate interventions including anterior-chamber irrigation, corneal endothelial transplantation or full-thickness keratoplasty shall be selected for optimal removal of cell masses. If cell aggregates or tumor-like lesions are detected in the posterior segment, pars plana vitrectomy will be performed to resect such masses, followed by specimen collection for pathological confirmation. Suitable surgical approaches will be chosen to

achieve maximal clearance of cell masses.

(2) Harms Related to Transplantation Therapy

Post-transplant ocular abnormalities including discomfort, pain, swelling and conjunctival hyperemia, iris injury, elevated intraocular pressure, infection, viral reactivation, progression of cataract and other acute adverse events associated with transplantation cannot be ruled out.

Situations Requiring Intervention-Infection and Viral Reactivation

Judgment criteria: Preliminary diagnosis is made based on sharp-rise anterior chamber cells, hypopyon, ocular discharge and conjunctival hyperemia. Confirmation is further achieved via metagenomic next-generation sequencing (mNGS) and microbial culture.

Management measures: The study principal investigator and operating physicians shall promptly discuss the therapeutic regimen and arrange hospitalization for the subject without delay. Intensive hourly topical administration of new-generation quinolones, cephalosporins, aminoglycosides and/or vancomycin eye drops shall be initiated. Anterior chamber irrigation and/or pars plana vitrectomy will be considered within the subsequent 24-hour period. Conjunctival sac swab culture is performed to identify causative pathogens. Antiviral therapy shall be instituted timely if viral infection is confirmed.

Cataract Progression

Judgment criteria: Significant progression of cataract is confirmed compared with the preoperative status.

Management measures: During the observation period, the study principal investigator and physicians performing regenerative-medicine-related interventions shall discuss the treatment plan. Cataract surgery combined with intraocular lens implantation will be considered if necessary.

Expulsive Hemorrhage

Terminate the transplantation therapy immediately. During hospitalization, the study principal investigator and operating physicians shall consult with relevant hospital specialists and administer appropriate additional surgical treatment.

Online-based meetings are acceptable for treatment-plan deliberations.

Follow-up Observations

Discomfort, pain, swelling, hyperemia, iris injury and other related conditions.

9. Evaluation and Reporting of Adverse Events

9.1 Definition of Adverse Events

An adverse event (AE) is any unfavorable or unintended sign,

symptom, or disease occurring in a subject regardless of its association with treatment such as allogeneic natural-killer-cell (NK-cell) transplantation.

Planned hospital admissions and examinations scheduled prior to initiation of this clinical study, as well as hospitalizations specified in the clinical study protocol shall not be regarded as adverse events.

Adverse events meeting the criteria below are defined as serious adverse events (SAEs):

- Resulting in death;
- Life-threatening;
- Requiring therapeutic hospitalization or prolongation of existing hospitalization;
- Resulting in permanent or substantial disability-incapacity;
- Causing congenital anomaly;
- Other serious events comparable to the conditions listed above.

9.2 Evaluation of Adverse Events

All adverse events identified from subject enrollment through study completion shall be evaluated in accordance with the provisions below. Clinically significant changes in laboratory-test

values relative to pre-transplant findings shall be reported as adverse events.

9.2.1 Onset Date of Adverse Events

The onset date is defined as the date when an adverse event is identified. For asymptomatic complications or incidental findings, the onset date shall be the date of definitive diagnosis (the date when diagnostic examinations are performed).

9.2.2 Severity Grading of Adverse Events

The study principal investigator and treating physicians shall assess all adverse events occurring throughout the clinical study in accordance with internationally accepted grading criteria (CTCAE and ASTCT consensus guidelines).

Grade 1: Mild adverse event requiring no intervention.

Grade 2: Moderate adverse event that necessitates minimal, local, or non-invasive intervention and interferes with instrumental activities of daily living.

Grade 3: Severe adverse event with substantial medical significance without immediate-life-threatening conditions; hospitalization or surgical intervention may be required, and basic activities of daily living are impaired.

Grade 4: Serious adverse event with acute-onset presentations that

result in death, are life-threatening, require hospitalization or prolonged-hospitalization duration, lead to permanent or substantial loss of function/disability, or demand urgent surgical management.

9.2.3 Outcome of Adverse Events

Outcomes of adverse events shall be categorized as described below.

Resolved	Symptoms and clinical findings disappear or improve, laboratory values normalize, or return to the pre-intervention levels.
Improved	The severity of symptoms and findings decreases by at least one grade; mild symptoms nearly resolve; or laboratory values recover to near pre-intervention levels.
Unresolved	Symptoms, findings or laboratory values remain unchanged; findings worsen on the final follow-up day during the observation period compared with onset; irreversible congenital anomalies develop; for deceased subjects where the adverse event is not a direct cause of death and the adverse event persists at the time of death; cases with sequelae even after partial resolution; partial improvement is achieved yet certain findings are confirmed as sequelae.
Death	A direct causal relationship between death and the adverse event is confirmed. “Confirmed direct correlation” means the adverse event causes death or substantially contributes to death. Adverse events presumed not to be the direct cause of death for the same subject shall not be classified as outcome “Death”.
Unknown	Follow-up in accordance with the clinical study protocol cannot be completed after transplantation owing to hospital transfer, relocation or other reasons.

9.2.4 Outcome Date of Adverse Events

Record the date of resolution, improvement, unresolved-status confirmation or death occurring during or after treatment administration. If the exact outcome date cannot be determined, document the date on which the outcome status is confirmed.

9.2.5 Causality Assessment between Adverse Events, Study-Related Procedures and Allogeneic NK-Cell Transplantation

Definitely related: There is a clear temporal association after transplantation, and evidence supporting a causal link is available.

Probably related: A clear temporal association post-transplantation exists, and alternative contributing factors such as underlying disease, comorbidities, concomitant medications and concomitant interventions can largely be ruled out.

Possibly related: A clear temporal association after transplantation is present. While other factors including underlying disease, comorbidities, concomitant medications and concomitant interventions are plausible contributors, the likelihood that the study treatment induced the adverse event cannot be excluded.

Unrelated: No temporal association is identified. The adverse event is inferred to arise from other factors such as underlying disease, comorbidities, concomitant medications and concomitant interventions.

9.3 Management upon Occurrence of Adverse Events

During the subject’s participation in the study, the study principal investigator and treating physicians shall perform necessary and appropriate examinations and monitoring to safeguard subject safety. When an adverse event develops, appropriate interventions shall be

provided. Specialists' consultations shall be arranged whenever needed to identify underlying causes.

Furthermore, adequate medical measures shall be administered for clinically problematic serious adverse events related to the study both during and after study participation.

After completion of the observation period, follow-up assessments within insurance-covered medical care may be performed if required from the perspective of safety assurance and scientific rationality. If the study principal investigator and treating physicians become aware of suspected illnesses, impairments, deaths or infections attributable to this transplant trial, they shall promptly report such findings to the administrator of the medical institution delivering the transplant therapy.

9.4 Documentation of Adverse Events

If new adverse events arise during the study, the items listed below shall be filled out in the adverse-event section of the case report form (CRF).

- Name of event
- Onset date
- Severity grade
- Outcome (Resolved, Improved, Unresolved, Death, Unknown)

- Outcome date
- Causality with study-related procedures and allogeneic NK-cell transplantation
- Management (study discontinuation or continuation, plus treatments administered for the adverse event)

9.5 Deficiencies of Allogeneic NK-Cells

Deficiencies of allogeneic NK-cells refer to quality deviations of cell products that may occur after release approval of cell products, during delivery to the operating room, as well as before and after administration to subjects. Specifically, if any of the following deficiencies of allogeneic NK-cells are confirmed, relevant contents shall be documented in the case report form (CRF):

(1) Presence of insoluble foreign substances;

(2) Occurrence of cell agglutination;

(3) Abnormal findings such as excessive air bubbles in the cell-product solution;

(4) The packaging container is not refrigerated and kept at room temperature;

(5) Damage to the packaging container;

(6) Residual excessive solution or precipitates inside the container

after transplantation;

(7) Abnormal temperature-monitoring records during transportation;

(8) Other unanticipated deviations.

Items to be Documented in the Case Report Form (CRF)

Item	Options or Contents
Transplantation performed	1. No; 2. Yes
Date of transplantation	
Date when the deficiency occurred	
Classification of deficiency	1. Material-related defect; 2. Defective product; 3. Quality deviation; 4. Others
Detailed description of the deficiency	
Occurrence of deficiency-related adverse events	1. No; 2. Yes (If yes, provide full details within the adverse-event documentation section)
Causal relationship between deficiency and adverse event	1. Unrelated; 2. Related; 3. Unknown

9.6 Reporting of Adverse Events and Deficiencies

Upon becoming aware of the adverse events mentioned below during the conduct of this clinical study, the study principal investigator and treating physicians shall promptly report the incidents to the administrator of the implementing medical-care institution.

10. Evaluation Endpoints

10.1 Safety Evaluation Endpoints

The primary safety endpoint consists of all adverse events including systemic manifestations.

Secondary safety endpoints include adverse events attributable to allogeneic NK-cell therapy and those resulting from transplantation-related surgery or procedures, mainly comprising elevated intraocular pressure, rejection reaction, ocular-surface or intraocular infection, cataract progression, expulsive hemorrhage and NK-cell proliferation. Judgement criteria shall be determined by the investigators.

In addition, considering adverse-reaction occurrences and abnormal changes in clinical examinations throughout this clinical study, the overall safety of each subject shall be categorized into the following four grades.

Safe: No adverse reactions and no abnormal shifts in clinical-laboratory findings during the study period.

Generally safe: Mild study-related adverse reactions occur and resolve spontaneously without targeted intervention; or clinically insignificant abnormalities in laboratory test results are observed, which return to normal upon follow-up examinations without intervention.

Questionable safety: Moderate study-related adverse reactions develop and can only be alleviated after symptomatic treatment; or laboratory abnormalities that require intervention resolve with follow-up

monitoring.

Unsafe: Severe study-related adverse reactions arise requiring drug discontinuation and specific treatment, even endangering the subject’s health; or marked abnormalities in laboratory parameters indicative of impaired organ-function, which demand immediate intervention or trial-drug withdrawal.

10.2 Efficacy Evaluation Items

10.2.1 Improvement of Corneal Epithelial Defect and Ocular-Surface Inflammation

Judgment method: At each study visit, the severity of inflammation and corneal epithelial defects shall be assessed under a slit-lamp biomicroscope. Improvement of 2 grades or higher relative to baseline assessment is defined as effective improvement in corneal-epithelial defect and ocular-surface inflammation.

Assessment criteria:

The therapeutic effect is considered effective if improvement is confirmed at no less than one visit before Visit 9 plus a total of two or more assessments including the final evaluation;

The outcome is deemed effective when improvement is observed on at least one occasion prior to Visit 9 and confirmed at the final assessment;

Conditions outside Case and will be regarded as ineffective.

10.2.2 Visual Acuity

Judgment criteria: At each study-visit time-point, visual acuity shall be measured using distance decimal visual-acuity values. A visual-acuity improvement is confirmed when the result improves by 2 grades or more compared with the baseline (pre-operative examination) assessment.

Assessment criteria:

The treatment is deemed effective if visual-acuity improvement is confirmed on no less than two visits (including the final evaluation), with at least one improvement recorded prior to Visit-9.

A beneficial effect is indicated if visual-acuity improvement occurs at least once before Visit-9 even though no improvement is identified at the final evaluation.

Cases outside scenarios and are regarded as ineffective.

10.2.3 Corneal Thickness Status

Judgment criteria: At each study-visit time-point, an improvement in corneal thickness is confirmed when the corneal thickness (μm) at the lesion site increases by more than 20 % compared with the pre-operative value.

Assessment criteria:

The outcome is considered effective when corneal-thickness improvement is confirmed on two or more visits including the final evaluation, with at least one confirmed improvement recorded prior to Visit 9.

A beneficial effect is indicated if improvement of corneal thickness is observed at least once before Visit 9, even though no improvement is noted at the final evaluation.

All conditions other than Case and shall be deemed ineffective.

Determination of insufficient therapeutic effect

Judgment criteria: After Visit 9, insufficient therapeutic effect is identified when the visual acuity is worse than the baseline (pre-operative visual acuity), and the corneal thickness at the ulcer site after treatment is lower than the baseline (pre-treatment corneal thickness).

11. Statistical Considerations

11.1 Target Sample Size and Rationale

This study is the-first-in-human research investigating allogeneic natural killer (NK)-cell transplantation for the treatment of Mooren’s ulcer. A minimal target sample size of 6 subjects is designated to confirm the reproducible safety profile of allogeneic NK-cell transplantation,

explore its efficacy and effective concentration against Mooren’s ulcer, and lay a foundation for subsequent clinical trials.

11.2 Demographic Characteristics

For the analysis of demographic characteristics, continuous variables shall be summarized with the number of subjects, mean value, standard deviation, maximum value, minimum value and median. Additional parameters for descriptive reporting will be determined as needed.

11.3 Safety and Efficacy Analysis

For continuous-type outcome indicators, the change from baseline at each assessment time-point shall be calculated, and descriptive statistics of measured values shall be summarized. For discrete-type outcome indicators, frequency summaries at each time-point or cross-tabulations of baseline and post-baseline measurements will be prepared. In addition, trend plots will be generated when necessary to assess time-dependent changes of indicators.

Definition for complete healing of Mooren’s ulcer: resolution of epithelial defect and clearance of stromal inflammatory-cell infiltration. Treatment response to cell transplantation is defined as complete ulcer healing without recurrence within 1-year follow-up. Ulcer recurrence is defined as the reappearance of epithelial defect accompanied by stromal infiltrationFrontiers

11.4 Interim Analysis

No interim analysis will be performed.

11.5 Deviations from the Original Statistical Plan

If analytical approaches different from those predefined in this clinical study protocol are adopted, all relevant revisions shall be documented in the final study report.

The handling of missing, excluded and outlier-data shall be determined case-by-case prudently after consultation with the Ethics Monitoring Committee.

12. Protocol Deviations

12.1 Deviations Resulting From Emergencies

If the principal investigator and study-attending physician fail to comply with this clinical research protocol to avert imminent risks to subjects or due to other medically necessary reasons, such facts shall be documented. The principal investigator shall promptly submit documents describing the incident and underlying reasons to the institutional administrator.

12.2 Deviations From Other Circumstances

Prior approval from the Ethics Committee shall be obtained in advance if the principal investigator intends to amend the clinical

research protocol. When protocol deviations occur for other reasons, the principal investigator shall immediately submit relevant documents with detailed descriptions and justifications to the institutional administrator.

12.3 Governing Guidelines

All personnel participating in this clinical research shall thoroughly study and understand and comply with the Declaration of Helsinki issued by the World Medical Association, Practicing Physician Law, Good Clinical Practice for Drugs, Regulation on the Administration of Clinical Research and Clinical Translation of New Biomedical Technologies and other applicable provisions governing human-subject-based medical research.

12.4 Protection of Subjects' Privacy

Regarding the protection of subjects' privacy, the following provisions shall be complied with. For subject-specific identity information obtained in connection with participation in this clinical study, linkable anonymization and administration shall be performed using the subject identification number assigned upon enrollment. Meanwhile, maximum efforts shall be made to protect subjects' privacy during preparation and retention of examination findings, observational assessments, evaluation results and pathological reports.

12.5 Conflict of Interest

The principal investigator and attending clinicians of this project shall manage potential conflicts of interest in compliance with conflict-of-interest guidelines specified in the Declaration of Helsinki by the World Medical Association, Good Clinical Practice for Drug Trials, Regulations on the Administration of Clinical Research and Clinical Translation and Application of New Biomedical Technologies and other relevant provisions.

12.6 Periodic Reporting

Administrators of the implementing medical institution shall submit reports concerning trial progress and related matters to the ethics committee.

12.7 Compliance

This clinical study shall be conducted in accordance with the study implementation plan and separately-specified standard operating procedures (SOPs). Any non-compliance incidents shall be documented in full detail.

Administrators of the implementing medical institution and the principal investigator shall abide by applicable clinical-trial-related regulations. They shall furnish the research protocol and implementation plan to the affiliated medical institution and ethics committee so that regular checks on proper study conduct can be performed. When

necessary, appropriate measures including study suspension and protocol amendments shall be adopted to ensure study compliance.

If personnel other than the principal investigator identify non-compliance issues, they shall report such findings to the principal investigator. Upon receiving such reports or discovering irregularities personally, the principal investigator shall document incident details and corresponding corrective actions taken. Reports shall be submitted to administrators of the cell-therapy-performing medical institution depending on the severity of the incident.

12.8 Processing, Documentation and Record-Retention of Data and Samples

After relevant documents generated from this clinical study are converted into electronic formats such as PDF, they shall be stored on a personal information-managed hard drive under the charge of the document-management officer.

13. Research Funding and Compensation

Allogeneic natural-killer (NK) cells will be provided to you free of charge. If you sustain injuries as a study subject resulting from the investigational product administration or from following study procedures properly under the guidance of study physicians, the sponsor shall compensate you for reasonable medical expenses incurred from

study-related injuries as well as appropriate damages required by applicable laws and regulations of the People’s Republic of China.

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