Exploratory Study on Small Extracellular Vesicles Derived from Human Umbilical Cord Mesenchymal Stromal Cells (hUC-MSC-sEV-001) Nasal Drops for Ischemic Stroke Patients

Project Informed Consent Form

Information Disclosure Page

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Dear Subject,

We sincerely invite you to participate in the "Exploratory Study on Small Extracellular Vesicles Derived from Human Umbilical Cord Mesenchymal Stromal Cells (hUC-MSC-sEV-001) Nasal Drops for Ischemic Stroke Patients" approved by Zhujiang Hospital of Southern Medical University. This study will be conducted at Zhujiang Hospital Affiliated to Southern Medical University, and it is expected that 6-18 subjects will voluntarily participate. The study has been reviewed and approved by the Ethics Committee of Zhujiang Hospital of Southern Medical University.

Your participation in this study is entirely voluntary, and you may choose not to participate. If you decide not to participate, this will not affect your relationship with your doctor. To help you make an informed decision about participating, it is important to understand the details of this study. Please read the following information carefully, and you may also discuss it with your family, friends, and doctor. If you have any questions or need more information about the study, please feel free to ask your doctor at any time.

1. Background and Objectives of the Study

The prevention and treatment of brain diseases is a major challenge under the "Healthy China" initiative. Mesenchymal stromal cells (MSCs) have made significant progress in the treatment of brain diseases, but they are limited by issues such as immune rejection and poor histocompatibility. MSC derivatives, such as exosomes or

small extracellular vesicles (sEVs), exhibit biological effects similar to MSCs. With low immunogenicity and the ability to cross the blood-brain barrier, sEVs have greater advantages in the treatment of brain diseases.

This study focuses on the therapeutic effects and mechanisms of four types of MSC-derived sEVs in ischemic stroke and Alzheimer's disease, and aims to advance their clinical research and application. It addresses key scientific questions, such as the mechanism by which MSC-derived sEVs regulate neuronal target cells and immune microenvironment cells in vivo to exert MSC-like functions. The study will provide a safe and effective MSC-sEV therapeutic protocol for solving global medical challenges like ischemic stroke and Alzheimer's disease.

Furthermore, this research will greatly promote the translational application of MSC-sEVs in treating other diseases, provide a scientific basis for the government to improve the treatment system for MSCs and their derivatives, enhance patient care standards, reduce mortality and disability rates, benefit a large number of patients, and create substantial economic and social benefits. It will also drive the rapid development of MSC-sEV applications, align with the national strategy of promoting innovation, and play a positive role in sustaining the growth of social and economic benefits.

2. Eligibility Criteria for Study Participants

(1) Inclusion Criteria

1. Diagnosed with ischemic stroke;

2. Aged 18-70 years, regardless of gender;

3. Anterior circulation occlusion confirmed by computed tomography angiography (CTA) or magnetic resonance angiography (MRA);

4. Modified Rankin Scale (mRS) score of 0-1 before the current stroke;

5. Alberta Stroke Program Early Computed Tomography Score (ASPECTS) ≥ 6 after stroke onset;

6. National Institutes of Health Stroke Scale (NIHSS) score ≥ 6 before enrollment;

7. Within 24 hours to 14 days after stroke onset;

8. No history of thrombolytic therapy or endovascular treatment;

9. No significant abnormalities in liver and kidney function: Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) ≤ 2.5 × upper limit of normal (ULN); serum creatinine and blood urea nitrogen ≤ 1.25 × ULN;

10. No significant abnormalities in cardiac function;

11. The subject or their legal representative can sign the informed consent form and comply with the study requirements for drug administration and follow-up.

(2) Exclusion Criteria

1. Intracranial hemorrhagic diseases confirmed by head CT: hemorrhagic stroke, epidural hematoma, subdural hematoma, intraventricular hemorrhage, subarachnoid hemorrhage, or hemorrhagic transformation of ischemic stroke;

2. Known severe hypersensitivity to contrast agents (excluding mild rash-like allergies);

3. Known bleeding tendency (including but not limited to): platelet count < 100×10^9/L; heparin administration within 48 hours with activated partial thromboplastin time (aPTT) ≥ 35 seconds; ongoing warfarin therapy with international normalized ratio (INR) > 1.7;

4. Concurrent brain tumors, epilepsy, or a history of severe traumatic brain injury;

5. Concurrent malignant tumors in other organ systems;

6. Concurrent severe systemic diseases such as immunodeficiency disorders and coagulation disorders;

7. Concurrent Alzheimer's disease, Parkinson's disease, or other neurodegenerative diseases that prevent completion of follow-up;

8. Severe local infection, systemic infection, immunocompromise, or ongoing use of immunosuppressants;

9. Positive hepatitis B surface antigen (HBsAg) or active infectious diseases;

10. History of allergic reactions to the study drug or drugs of similar composition;

11. Known allergic diathesis;

12. Nasal structural abnormalities or nasal lesions;

13. Cerebrospinal fluid rhinorrhea;

14. Participation in other clinical trials within 3 months prior to screening;

15. Unwillingness or inability to comply with protocol-specified procedures;

16. Pregnant or lactating women, or women of childbearing potential who cannot or refuse to take appropriate contraceptive measures;

17. Poor compliance with clinical trial requirements (e.g., uncontrolled psychiatric disorders);

18. Other conditions deemed unsuitable for enrollment by the investigator.

(3) Records of Failed Screening

1. Investigators are responsible for all subjects who have signed the informed consent form.

2. If a subject is deemed ineligible during screening (at the first visit), the investigator shall complete the Case Report Form (CRF) and document the reason for screening failure. The screening number assigned to the ineligible subject shall not be reused.

(4) Criteria for Determining Lost-to-Follow-Up Subjects

A subject will be considered lost to follow-up if:

1. They experience adverse events (AEs), serious adverse events (SAEs), other complications, or special physiological changes, and the investigator judges that study participation should be terminated and appropriate treatment provided;

2. Their condition of concurrent diseases (excluding stroke) deteriorates within a certain period, requiring alternative treatment to protect the subject (they will be withdrawn from the trial); however, if the subject's stroke condition deteriorates after receiving hUC-MSC-sEV-001 and is no longer suitable for continuing the study, the investigator shall provide timely treatment, and the subject shall withdraw from the study (this case will be counted as an invalid case, not a lost-to-follow-up case);

3. They have poor compliance (e.g., failure to receive required treatment/follow-up or refusal to continue the clinical study);

4. They withdraw from the study midway or are lost to follow-up: the investigator shall take active measures to complete the final assessment (if possible) for efficacy and safety analysis, and provide corresponding treatment. The reason for withdrawal shall be documented in the CRF for all lost-to-follow-up subjects.

(5) Provisions for Excluded Cases

A case will be excluded if:

1. It is a misdiagnosed case;

2. The subject meets the inclusion criteria but is enrolled but never receives hUC-MSC-sEV-001 treatment, or fails to receive scheduled administration due to hUC-MSC-sEV-001 production failure;

3. The subject participates in other clinical trials during the study;

4. The subject receives unapproved combined medications or symptomatic treatments (especially drugs or therapies that significantly affect study results), which interfere with the judgment of efficacy and safety;

5. The subject receives hUC-MSC-sEV-001 treatment but has no evaluable records or severely missing evaluation indicators;

6. The reason for exclusion shall be explained, and the CRF shall be retained for verification. The inclusion of excluded cases in the statistical analysis set shall be determined through discussion in the data verification meeting. Subjects who receive at least one dose of treatment and have safety records may be included in the safety analysis as appropriate.

(6) Criteria for Terminating or Withdrawing from the Study

1. Study Termination: Refers to the early cessation of the entire study before completion in accordance with the protocol. The primary purpose of study termination is to protect the rights and interests of subjects, ensure trial quality, and avoid unnecessary economic losses.

2. Investigator-Determined Subject Withdrawal:

① During the study, the subject develops a disease or special physiological change that makes them unsuitable for continuing the trial;

② The subject uses other drugs that affect the efficacy evaluation of hUC-MSC-sEV-001 (e.g., antineoplastic drugs, neurotrophic drugs) during the study;

③ The subject experiences an allergic reaction due to hUC-MSC-sEV-001 administration, and the investigator determines that trial termination is necessary;

④ The subject has poor compliance, which affects the safety and efficacy evaluation of hUC-MSC-sEV-001.

3. Subject-Initiated Withdrawal:

① The subject is unwilling or unable to continue the clinical study for any reason and requests to withdraw from the trial;

② The subject does not explicitly request withdrawal but stops receiving hUC-MSC-sEV-001 treatment or related examinations and is lost to follow-up (also considered "withdrawal").

For subjects who withdraw midway, the investigator shall take active measures to contact the subject for a return visit and complete the final assessment (if possible) for efficacy and safety analysis. The reason for withdrawal (if provided) shall be documented in the CRF. For subjects who withdraw due to allergic reactions or adverse events, the investigator shall provide corresponding treatment based on the subject's actual condition.

(7) Criteria for Terminating the Study

The study will be terminated immediately if:

1. A serious adverse event occurs during the study;

2. hUC-MSC-sEV-001 production fails, making it impossible to administer scheduled doses to subjects;

3. hUC-MSC-sEV-001 is found to have no clinical value during the study (to avoid delaying effective treatment for subjects and unnecessary economic losses);

4. A major flaw is found in the clinical study protocol (making it difficult to evaluate the efficacy of hUC-MSC-sEV-001), or a well-designed protocol has serious deviations during implementation (making it difficult to evaluate efficacy);

5. The sponsor requests termination due to funding, management, or other reasons;

6. The study is required to be terminated by the National Medical Products Administration (NMPA).

3. Study Procedures and Methods

Before enrolling in this study, the doctor will take your medical history and conduct relevant examinations. If you meet the eligibility criteria, you may voluntarily participate in the study and sign the informed consent form. If you refuse to participate, we will continue your original treatment plan.

If you meet the inclusion criteria and agree to participate in this study, you will receive MSC-sEV preparation treatment in addition to standard ischemic stroke treatment.

A traditional "3+3" dose-escalation design will be adopted. You may be assigned to one of three dose groups (2.8×10^{10} units, 1.4×10^{11} units, 7.0×10^{11} units per administration, once daily for 4 consecutive days via intranasal instillation). Each dose group initially enrolls 3 subjects, with sequential enrollment. If no dose-limiting toxicity (DLT) is observed in 3 subjects, the next dose level can be explored. If 1 out of 3 subjects experiences DLT, an additional 3 subjects will be enrolled in the same dose group. The next dose level can be explored only if no DLT is observed in the additional 3 subjects. A total of 6 to 18 subjects will be recruited during the dose-escalation phase.

You will receive hUC-MSC-sEV-001 nasal drops on the basis of standard treatment (nasal mucosa pretreatment with hyaluronidase: 150 U per nostril before each instillation). Intranasal instillation of hUC-MSC-sEV-001 will be administered 4 times: once on the day of enrollment, and once on Days 2, 3, and 4 after enrollment.

4. Requirements for Subject Cooperation

You agree to participate in all required examinations, treatments, and follow-up visits in accordance with the study protocol. You must provide true information about your medical history and current health status; report any discomfort experienced during the study to the research doctor; and avoid using any drugs (including over-the-counter drugs) or receiving other treatments without consulting your research doctor (as certain drugs/treatments may be prohibited during the study). Please provide detailed information about any drugs or treatments you may be using, and your doctor will discuss with you which drugs or treatments to avoid if you participate in this study. Additionally, please inform the research doctor if you have participated in other studies recently or are currently participating in other studies.

If you plan to become pregnant, you should not participate in this study. You must ensure no pregnancy occurs during the study period. If you become pregnant accidentally, please immediately inform the research doctor, and discuss with the investigator whether to terminate the pregnancy promptly and withdraw from the study. If you decide to terminate the pregnancy, you will bear the associated costs; if you decide to continue the pregnancy, you will assume the potential risks.

Furthermore, you need to note the following during the treatment period:

- If your condition deteriorates during the study, you may withdraw from the study and receive other treatments. You should cooperate with the doctor to complete relevant examinations and evaluations as much as possible, and your study data will be treated as invalid.

- Records of Combined Medications: If other medications are required for other reasons, the doctor will document the reason for use, generic name, dosage form, dosage and administration, start date, and stop date of the combined medication in the "Combined Medications" section of the CRF (the solvent for injectable preparations does not need to be recorded). The doctor will carefully record all combined medications you use during the clinical study (from the signing of the informed consent form to the end of the study).

- Records of Combined Treatments: During the clinical study (from the signing of the informed consent form to the end of the study), the doctor will document all treatment measures you receive in the CRF.

- Records of Rehabilitation Therapy: During the clinical study (from the signing of the informed consent form to

the end of the study), the doctor will document all rehabilitation therapy you receive in the CRF.

- Prohibited and Cautiously Used Drugs/Treatments: You should use antibiotics and antiviral drugs with caution within 6 months after enrollment; use immunosuppressants and antidepressants with caution during the study; and avoid using antineoplastic drugs, neurotrophic drugs, acupuncture, and hyperbaric oxygen therapy during the study.

The specific items you need to cooperate with are as follows:

First Visit [Baseline (Enrollment Day)]

The following tasks will be completed during this visit:

1. Sign the informed consent form;

2. Collect demographic and disease data;

3. Conduct relevant examinations:

- For outpatients: Measure vital signs and collect blood/urine samples for tests (complete blood count [CBC], urinalysis, pregnancy test, liver and kidney function, glucose metabolism, lipid metabolism, coagulation function [four items], serum electrolytes, infectious disease screening [8 items], tumor markers) in the outpatient department; then undergo chest X-ray, electrocardiogram (ECG), head and neck CTA, computed tomography perfusion (CTP), and brain magnetic resonance imaging (MRI) plain scan in the corresponding examination departments.

- For inpatients: Measure vital signs and collect blood/urine samples for the above tests in the ward; then undergo the above imaging examinations in the corresponding departments.

4. Complete scale assessments by the neurosurgery or rehabilitation department: NIHSS, Modified Barthel Index, EQ-5D-5L score, mRS score;

5. The investigator reviews the inclusion and exclusion criteria item by item;

6. Perform nasal mucosa pretreatment with hyaluronidase (150 U per nostril), then administer hUC-MSC-sEV-001 nasal drops once (within 24 hours of enrollment). Administer hUC-MSC-sEV-001 nasal drops once on Days 2, 3, and 4 after enrollment (48h, 72h, 96h after enrollment) following hyaluronidase pretreatment. Record the patient's vital signs after each treatment;

7. Document combined medications.

Second Visit (7 ± 1 Days After Enrollment)

The following tasks will be completed during this visit:

1. Record the subject's vital signs;

2. Measure vital signs and collect blood/urine samples for tests (CBC, urinalysis, liver and kidney function, glucose metabolism, lipid metabolism, coagulation function [four items], serum electrolytes, tumor markers) in the ward;

3. Document combined medications, treatment measures, rehabilitation therapy, and adverse events;

4. Complete scale assessments by the rehabilitation department: NIHSS, Modified Barthel Index, EQ-5D-5L score, mRS score.

Third Visit (14 ± 2 Days After Enrollment)

The following tasks will be completed during this follow-up visit:

1. Record the subject's vital signs;

2. Conduct relevant examinations: Measure vital signs and collect blood/urine samples for tests (CBC, urinalysis, liver and kidney function, glucose metabolism, lipid metabolism, coagulation function [four items], serum electrolytes, infectious disease screening [8 items], tumor markers); then undergo chest X-ray, ECG, head and neck CTA, CTP, and brain MRI plain scan;

3. Complete scale assessments by the rehabilitation department: NIHSS, Modified Barthel Index, EQ-5D-5L score, mRS score;

4. Document combined medications;

5. Schedule the next visit.

Fourth Visit (90 ± 14 Days After Enrollment)

The following tasks will be completed during this visit:

1. Record the subject's vital signs;

2. Conduct relevant examinations: Measure vital signs and collect blood/urine samples for tests (CBC, urinalysis, liver and kidney function, glucose metabolism, lipid metabolism, coagulation function [four items], serum electrolytes, infectious disease screening [8 items], tumor markers); then undergo chest X-ray, ECG, head and neck CTA, CTP, and brain MRI plain scan;

3. Complete scale assessments by the rehabilitation department: NIHSS, Modified Barthel Index, EQ-5D-5L score, mRS score;

4. Document combined medications.

5. Benefits of Participating in This Study

Direct Benefits

If you participate in this study, you will receive MSC-sEV preparation treatment in addition to standard treatment. It is expected that this may further improve your neurological deficit symptoms. However, the preparation is currently in the clinical research stage, and it may not achieve the expected efficacy in improving neurological deficits.

Potential Benefits

The information obtained from your participation in this study is expected to benefit future patients with the same condition as yours.

Please note that you may not receive any direct benefits during your participation in the study.

6. Potential Risks and Discomforts

Participating in this study may expose you to the following potential risks:

1. Risks Related to the MSC-sEV Preparation

1.1 Allergic reactions may occur during the administration of the MSC-sEV preparation;

1.2 Bacterial/viral contamination of the MSC-sEV preparation may occur (e.g., during processing or transportation);

1.3 Most of the MSC-sEV preparation may be retained by other organs, with only a small portion exerting therapeutic effects.

2. Risks Related to Examinations During Treatment

2.1 Blood sample collection: You may experience slight discomfort during blood collection. Possible side effects include dizziness, phlebitis, pain, bruising, or bleeding at the puncture site. There is also a small risk of infection;

2.2 ECG: Skin irritation is rare, but it may occur due to the electrodes or gel used during the ECG examination.

3. Other Unpredictable Risks During Treatment

All the above risks may lead to serious consequences, including life-threatening situations.

7. Treatment and Compensation for Study-Related Harm

The project undertaking unit will compensate for trial-related harm in accordance with Chinese laws and regulations. Compensation includes free medical treatment for the harm and statutory compensation.

In addition, clinical trial insurance has been purchased for this study to provide compensation coverage for potential study-related harm.

In accordance with applicable laws and regulations, the following situations resulting in harm to you are not covered by the aforementioned free medical treatment, and the project undertaking unit will not provide compensation. Please read carefully:

1. Natural progression of your underlying diseases (including pre-existing diseases and the target disease of this treatment), or complications and related harm caused thereby;

2. Harm caused by your failure to comply with this informed consent form, the doctor’s advice, or instructions;

3. Self-harm caused by any intentional behavior of yours;

4. Harm caused by force majeure. Force majeure includes but is not limited to natural disasters (earthquakes, typhoons, floods, etc.), government actions (expropriation or requisition), wars, etc.

8. Study-Related Examinations and Costs

You will be required to cooperate with completing relevant examinations for this project, and the costs of these examinations will be covered by the study.

The study-related examinations include: CBC, liver and kidney function, coagulation function (four items), serum electrolytes, urinalysis, glucose metabolism, lipid metabolism (these 7 items will be conducted 4 times); CTA, CTP, MRI, tumor markers, chest X-ray, ECG (these 6 items will be conducted 3 times); infectious disease screening (8 items, conducted 2 times). For female subjects, a urine pregnancy test will also be included (only for women of childbearing age or premenopausal women, conducted 1 time).

Completing these examinations will require collecting a small amount of blood samples (approximately 4-10 milliliters per follow-up visit). We promise that the collected blood samples will only be used for these examinations, and you will not receive financial compensation for blood collection.

The following table lists the examinations covered by the study (free of charge):

Examination Item	**Cost per Session (RMB)**	**Number of Sessions**	**Total Cost (RMB)**
Complete Blood Count (CBC)	18.4	4	73.6
Urinalysis	29.44	4	117.76
Liver and Kidney Function	155.48	4	621.92
Glucose Metabolism	79.12	4	316.48
Lipid Metabolism	73.6	4	294.4
Coagulation Function	84.64	4	338.56
Serum Electrolytes	27.60	4	110.4

Examination Item	Cost per Session (RMB)	Number of Sessions	Total Cost (RMB)
Tumor Markers	253.92	3	761.76
Head and Neck CTA	1389.16	3	4167.48
Cerebral CTP	936.45	3	2809.35
Brain MRI	840	3	2520
Urine Pregnancy Test (Female Subjects)	6.44	1	6.44
Infectious Disease Screening (8 Items)	322	2	644
Chest X-ray (DR)	72.5	3	217.5
Electrocardiogram (ECG)	27.08	3	81.24
Total			**13,080.89**

You will bear the costs of your standard treatment.

If you require additional examinations or medical care unrelated to this study, you will bear the associated costs.

Treatment and examinations for other concurrent diseases will not be covered by the study.

This study does not involve commercial development, but the sponsor has the right to store and use the existing study data and documents in accordance with the study agreement.

9. Privacy and Confidentiality

Your medical information will be kept confidential at all times. In addition to your research doctor, government regulatory authorities and the Ethics Committee may review your study-related original medical records to ensure the study is conducted in compliance with regulations and the data is authentic and reliable. However, all information will remain confidential. The study results may be published in medical journals, but your identity will not be disclosed.

10. Approval Status of Human Genetic Resources

This project does not involve human genetic resources.

11. Contact Information

If you have any concerns, questions, or encounter any emergencies related to the study, please contact your doctor promptly. Please keep this information for future reference:

Doctor's Name (Printed): _____

Office Phone: _____

Mobile Phone: _____

If you have questions about your rights as a subject, you may contact the Medical Ethics Committee of Zhujiang Hospital of Southern Medical University during working hours on national working days:

Phone: 020-62783254

12. Additional Information

Your participation in the study is voluntary. You may refuse to participate or withdraw from the study at any stage without discrimination or retaliation, and your medical treatment and rights will not be affected. You may

withdraw your informed consent at any time during the study. If you wish to withdraw your consent, please call Dr. Zhang at 15820205853.

After you voluntarily withdraw from the study, the investigator has the right to process the study data generated before your withdrawal, and all processing will comply with the "Privacy" requirements specified in Section 8 of this informed consent form.

Consent Signature Page

Subject's Statement

I have read the above content and understand the nature, purpose, risks, and benefits of this study. I have had the opportunity to discuss this study with the doctor and ask questions, and all my questions have been answered satisfactorily.

I agree to comply with the requirements for subjects and fully cooperate with the research team. I will truthfully and objectively provide information about my health status before participation, during the study, and at each follow-up visit.

I understand that participation in this study is voluntary, and I confirm that I have had sufficient time to consider this decision. I also understand that I may withdraw from the study at any time without any adverse impact on my subsequent treatment, and that the investigator has the right to terminate my study participation based on my condition.

I hereby consent to participate in this clinical study. This informed consent form is made in duplicate, and I will receive a signed and dated copy.

Subject's Signature: _____

Contact Phone: _____

Date: _____

(If Applicable) Legal Representative/Guardian's Signature

Signature: _____

Relationship with the Subject: _____

Contact Phone: _____

Note: For subjects unable to express consent, the above information shall be provided to their legal representative/guardian.

(If Applicable) Witness's Signature

Signature: _____

Contact Phone: _____

Date: _____

Note: If the subject or their guardian is unable to read, the witness shall read the informed consent form and other written materials to them and witness the informed consent process.

Investigator's Statement

I have explained the details of this study to the above subject and provided them with a signed copy of the informed consent form.

Investigator's Signature: _____

Contact Phone: _____

Date: _____