Exploratory Study of Intranasal Administration of Human Umbilical Cord Mesenchymal Stem Cell-Derived Extracellular Vesicles for the Treatment of Ischemic Stroke

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Summary of the research plan

Brief Title		Exploratory study on the efficacy and safety of small extracellular vesicles from human umbilical cord-derived mesenchymal stromal cells nasal drops for ischemic stroke patients
Sponsor		Zhujiang Hospital of Southern Medical University
Study Duration		November 2025 - November 2027
Objectives		Primary objective: To evaluate human umbilical cord mesenchymal stem cell-derived small extracellular vesicles hUC-MSC-sEV-001 nasal drops Safety and preliminary effectiveness in the treatment of patients with ischemic stroke.
Study design		Single-center, prospective, dose-finding clinical trial
Eligibility	Inclusion criteria	1. Ischemic Stroke 2. Age 18-70 years, no gender restriction 3. Confirmed anterior circulation occlusion by CTA or MR angiography (MRA) 4. Pre-stroke mRS score of 0-1 5. Post-stroke ASPECTS score >= 6 6. Pre-enrollment NIHSS score>= 6 7. Within 24 hours to 14 days of stroke onset 8. Patients who have not received thrombolysis or endovascular treatment 9. No significant liver or kidney dysfunction: ALT and AST < = 2.5 times the upper limit of normal, serum creatinine and blood urea nitrogen < = 1.25 times the upper limit of normal 10. No significant cardiac dysfunction 11. The subject or legal representative can sign the informed consent form and comply with the requirements of the study for administration and follow-up.
	Exclusion criteria	1. Intracranial hemorrhagic conditions observed on cranial CT: hemorrhagic stroke, epidural hematoma, subdural

		hematoma, intraventricular hemorrhage, subarachnoid hemorrhage, or hemorrhagic transformation, etc. 2. Known severe allergy to contrast agents (excluding mild rash-type allergies) 3. Known bleeding tendencies (including but not limited to): platelet count < 100×10^9/L; received heparin treatment within 48 hours with aPTT >= 35s; currently taking warfarin with INR > 1.7 4. Patients with brain tumors, or a history of epilepsy and severe head trauma 5. Patients with other systemic malignancies 6. Patients with other serious systemic diseases such as immunodeficiency, coagulation disorders, etc. 7. Patients with Alzheimer's disease, Parkinson's disease, or other neurodegenerative diseases that prevent them from completing follow-up 8. Patients with severe local infection, systemic infection, immunodeficiency, or those currently taking immunosuppressants 9. Patients who are positive for hepatitis B surface antigen or currently suffering from other infectious diseases 10. Patients who have allergic reactions to the drug used in this study or similar drugs 11. Patients with known allergic constitution 12. Patients with nasal structural abnormalities or lesions 13. Patients with cerebrospinal fluid rhinorrhea 14. Patients who have participated in other clinical trials within the past 3 months 15. Unwilling or unable to comply with the procedures specified in the protocol 16. Pregnant or breastfeeding women, or women of childbearing potential who are unwilling or unable to use appropriate contraceptive measures 17. Patients clearly lacking the compliance to complete the clinical trial, such as those suffering from uncontrolled mental illness 18. Other situations deemed unsuitable for enrollment by the researchers
Experimental drug and Treatment Plan		Experimental drug: hUC-MSC-SEV-001 nasal drops derived from small extracellular vesicles of human umbilical cord mesenchymal stem cells Treatment plan: On the basis of conventional treatment, hUC-MSC-SEV-001 nasal drops will be administered: hUC-MSC-SEV-001 will be administered via nasal drip for a total of 4 times, with a dosage of 1 × 10 * 11articles per

		dose. The patient will receive one dose on the day of enrollment, and another dose of hUC-MSC-SEV-001 will be administered on the 2nd, 3rd, and 4th days after enrollment.
Study design		Using a traditional "3+3" dose-escalation design, subjects will be sequentially assigned to one of three dose groups (2.8×10^{10} units, 1.4×10^{11} units, and 7.0×10^{11} units per dose, once daily, with continuous intranasal instillation for 4 days). Each dose group will include 3 subjects, who will be enrolled one by one. The exploration of the next dose can only proceed if no dose-limiting toxicity (DLT) is observed in the 3 subjects. If 1 out of the 3 subjects experiences DLT, an additional 3 subjects will be enrolled in the same dose group; the exploration of the next dose can only move forward if no DLT is observed in these newly added 3 subjects. A minimum of 6 and a maximum of 18 subjects will be recruited during the dose exploration phase. Patients will receive other standard medical treatments in accordance with the recommendations in the *2022 Chinese Guidelines for Secondary Prevention of Ischemic Stroke and Transient Ischemic Attack*, including antiplatelet drugs or anticoagulants, antihypertensive drugs, lipid-lowering drugs, and glucose-lowering drugs.
Outcome Measures	Primary Outcome Measure	Dose-Limiting Toxicity (DLT) Related to hUC-MSC-sEV-001 Includes: (1) Grade 3-4 severe adverse events occurring on day 14 (+-2), graded according to Common Terminology Criteria for Adverse Events （CTCAE） V5.0; (2) NIHSS score increase of ≥4 from baseline on day 14 (+-2); NIHSS score assessed by a professional rehabilitation physician;(3) Symptomatic intracranial hemorrhage occurring on day 14 (+-2); detected via CT scan and patient symptom presentation.
	Secondary Outcome Measures:	Serious adverse events related to the investigational drug within 90 (+-7) days. Distribution of Modified Rankin Scale (mRS) Scores within 90 (+-7) days. Barthel Index within 90 (±7) days. Five-level, five-dimensional EuroQol (EQ-5D-5L) . Proportion of Modified Rankin Scale (mRS) Scores of 0-2 within 90 (+-7) days. All-cause mortality at 90 (+-7) days. Changes in infarct volume at 14 (+-2) days compared to baseline. Change from Baseline in National Institutes of Health Stroke Scale (NIHSS) Score.
Follow-up duration		Baseline (0~1 day), 7 (±1) days, 14 (±2) days, 90 (±7) days
Sample size		6-18 subjects need to be included.

Statistical Analysis Methods	The primary objective of this study is to evaluate the safety and preliminary efficacy of hUC-MSC-sEV-001 nasal drops (small extracellular vesicles derived from human umbilical cord mesenchymal stem cells) in the treatment of acute ischemic stroke. Baseline information will be described based on the distribution type of variables: continuous variables will be presented as mean ± standard deviation, as well as P50, P25, and P75 percentiles; categorical variables will be described using frequency (percentage). For inter-group differences: analysis of variance (ANOVA) or Kruskal-Wallis rank sum test will be used for continuous variables; chi-square test or Fisher's exact test will be used for categorical variables. A generalized linear model will be applied to analyze the difference in the proportion of patients with a modified Rankin Scale (mRS) score of 0-2 at 90 days (±7 days) between the two groups.

Research Background

The prevention and treatment of brain diseases is a major challenge for "Healthy China". While stem cells have made significant progress in the treatment of brain diseases, they have limitations such as immune rejection and poor histocompatibility. Stem cell derivatives, such as exosomes or small extracellular vesicles (sEVs), exhibit similar therapeutic effects to stem cells. Moreover, their low immunogenicity and ability to easily cross the blood-brain barrier give them greater advantages in the treatment of brain diseases.

This research focuses on the therapeutic effects and mechanisms of four types of stem cell-derived sEVs in ischemic stroke and Alzheimer's disease (AD), with the goal of advancing their clinical research and application. The study addresses key scientific issues, including the mechanism by which stem cell-derived sEVs regulate neuronal target cells and immune microenvironment cells in vivo to achieve stem cell-like functions. It will also provide a safe and efficient mesenchymal stem cell-derived sEV (MSC-sEV) therapeutic protocol for solving global challenges such as ischemic stroke and AD.

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

Main Research Content

Based on adherence to the Good Clinical Practice (GCP) principles for national clinical trial research and the standardized procedures of Contract Research Organizations (CROs), this study aims to evaluate the safety and preliminary efficacy of hUC-MSC-sEV-001 in the treatment of ischemic stroke by enrolling a small sample population. Additionally, the study will explore and determine an appropriate dose to lay the foundation for subsequent research.

Research Methods

Clinical-grade hUC-MSC-sEV-001 produced under Good Manufacturing Practice (GMP) conditions will be obtained, and standardized procedures compliant with clinical research requirements will be established, covering aspects such as production, testing and identification, storage and resuscitation, transportation and packaging, and quality control.

Clinical research protocols, investigator's brochures, case report forms (CRFs), informed consent forms (ICFs), and recruitment advertisements will be developed, followed by submission for approval to the Ethics Committee (EC).

In accordance with the research protocol, 6–18 volunteers with ischemic stroke will be recruited to preliminarily explore the safety of hUC-MSC-sEV-001 in the treatment of cerebral ischemia. The optimal therapeutic and safe dose derived from animal experiments will be converted, and the corresponding treatment route and frequency will be determined. Safety assessments—including changes in vital signs and adverse reactions after treatment—will be conducted at baseline (Day 0–1), Day 7 (±1), Day 14 (±2), and Day 90 (±7). Additionally, efficacy will be evaluated from the perspectives of laboratory indicators, imaging findings, neurological function assessment indicators, and follow-up data.

1. Study Design

(1) Overall Study Design

Single-center, prospective, dose-escalation clinical trial.

(2) Trial Administration Strategy

A traditional "3+3" dose-escalation design will be adopted. Subjects will be sequentially assigned to one of three dose groups (2×10^{10} units, 1×10^{11} units, 5×10^{11} units per administration, once daily for 4 consecutive days via intranasal instillation). Each dose group initially enrolls 3 subjects, with sequential enrollment (i.e., the next subject is enrolled only after the previous subject completes the 14-day DLT observation period).

- If no DLT is observed in 3 subjects of a dose group, the study proceeds to the next higher dose level.

- If 1 out of 3 subjects experiences DLT, an additional 3 subjects are enrolled in the same dose group. If no DLT is observed in the additional 3 subjects, the study proceeds to the next higher dose level; if ≥1 additional subject experiences DLT, the current dose level is defined as the maximum tolerated dose (MTD), and dose escalation is terminated.
- A total of 6 to 18 subjects will be recruited during the dose-escalation phase.

(3) Study Suspension or Early Termination

The study will be suspended or terminated early if any of the following occurs:

- Violation of the *Good Clinical Practice (GCP)* or the *Administrative Measures for Stem Cell Clinical Research (Trial)*;
- Emergence of new safety or efficacy data on MSC-sEVs indicating that the trial poses a serious risk to patient safety or is clearly ineffective;
- Failure to enroll subjects as scheduled for 3 consecutive months;
- Major protocol deviations that affect the validity of study data;
- Request for termination by the sponsor or regulatory authorities.

2. Subject Selection Criteria

(1) Inclusion Criteria

- Confirmed diagnosis of acute ischemic stroke (in accordance with the *Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke (2023)*);
- Aged 18-70 years, regardless of gender;
- Anterior circulation occlusion (internal carotid artery, middle cerebral artery M1/M2 segment) confirmed by CTA or MRA;
- mRS score of 0-1 before the current stroke episode (indicating no or minimal disability prior to the index event);
- ASPECTS ≥ 6 on non-contrast computed tomography (NCCT) or diffusion-weighted imaging (DWI) after stroke onset (indicating a small to moderate infarct volume);
- NIHSS score ≥ 6 at screening (indicating moderate to severe neurological deficit);
- Time from stroke onset to screening enrollment: 24 hours to 14 days;
- No history of thrombolytic therapy (e.g., alteplase, tenecteplase) or endovascular treatment (e.g., mechanical thrombectomy, angioplasty) for the current stroke;

- No significant abnormalities in liver and kidney function: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 × upper limit of normal (ULN); serum creatinine (Scr) and blood urea nitrogen (BUN) ≤ 1.25 × ULN;
- No significant cardiac dysfunction (New York Heart Association [NYHA] heart failure classification ≤ II, no recent myocardial infarction within 6 months);
- The subject or their legal representative is able to understand and sign the informed consent form, and agrees to comply with the study visit schedule and follow-up requirements.

(2) Exclusion Criteria

- Intracranial hemorrhagic diseases confirmed by head CT/MRI: hemorrhagic stroke, epidural hematoma, subdural hematoma, intraventricular hemorrhage, subarachnoid hemorrhage, or hemorrhagic transformation of ischemic stroke;
- Known severe hypersensitivity to contrast agents (e.g., anaphylactic shock, angioedema; excluding mild maculopapular rash);
- Known bleeding diathesis (including but not limited to): platelet count < 100 × 10^9/L; heparin administration within 48 hours with aPTT ≥ 35 seconds; ongoing warfarin therapy with INR > 1.7; use of direct oral anticoagulants (DOACs) within 72 hours prior to screening;
- Concurrent primary or metastatic brain tumors, a history of epilepsy with active seizures within 1 year, or a history of severe traumatic brain injury with persistent neurological sequelae;
- Malignant tumors involving other organ systems (except for basal cell carcinoma of the skin or in situ cervical cancer with curative treatment and no recurrence for ≥5 years);
- Other severe systemic diseases, such as primary immunodeficiency disorders, acquired immunodeficiency syndrome (AIDS), severe coagulation disorders (e.g., hemophilia), or decompensated cirrhosis;
- Alzheimer's disease, Parkinson's disease, or other neurodegenerative diseases that prevent completion of neurological function assessments and follow-up;
- Severe local infection (e.g., nasal cellulitis), systemic infection (e.g., sepsis), or immunocompromise (e.g., long-term use of corticosteroids ≥10 mg/day prednisone equivalent for >1 month);
- Positive HBsAg, positive hepatitis C virus (HCV) antibody with detectable viral load,

positive human immunodeficiency virus (HIV) antibody, or active syphilis (positive treponema pallidum particle agglutination assay [TPPA] with positive rapid plasma reagin [RPR] test);

- History of allergic reactions to hUC-MSC-sEVs, hyaluronidase, or excipients in the study drug;
- Known atopic diathesis (e.g., severe asthma, urticaria requiring systemic treatment);
- Nasal structural abnormalities or lesions that affect drug delivery (e.g., severe nasal septal deviation, nasal polyps, chronic sinusitis with acute exacerbation);
- Cerebrospinal fluid rhinorrhea (spontaneous or post-traumatic);
- Participation in other interventional clinical trials within 3 months prior to screening;
- Unwillingness or inability to comply with protocol-specified procedures (e.g., refusal to undergo required imaging examinations);
- Pregnant or lactating women (confirmed by urine pregnancy test for women of childbearing potential); women of childbearing potential who cannot or refuse to use effective contraceptive measures (e.g., combined oral contraceptives, intrauterine device) during the study and for 3 months after the last dose;
- Uncontrolled psychiatric disorders (e.g., schizophrenia, major depressive disorder with suicidal ideation) that affect compliance;
- Other conditions deemed unsuitable for enrollment by the investigator (e.g., terminal illness with life expectancy <6 months).

(3) Records of Failed Screening

- Investigators are responsible for all subjects who have signed the informed consent form, regardless of whether they are ultimately enrolled.
- If a subject is deemed ineligible during screening (at the first visit), the investigator shall complete the "Screening Failure Form" (attached to the CRF) and document the specific reason for failure (e.g., "NIHSS score = 4 < 6", "History of mechanical thrombectomy"). The screening number assigned to the ineligible subject shall not be reused.

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

A subject is considered lost to follow-up if:

- They experience an AE, SAE, or other complication that requires study termination and urgent treatment, as judged by the investigator;

- Their condition of a concurrent disease (excluding the index stroke) deteriorates, requiring alternative treatment that conflicts with the study protocol; if the subject's stroke condition deteriorates after receiving hUC-MSC-sEV-001 (e.g., progression of neurological deficit), they shall withdraw from the study and be classified as an invalid case (not a lost-to-follow-up case);
- They have poor compliance (e.g., missed ≥2 scheduled visits, refusal to undergo required assessments) or express unwillingness to continue the study;
- They are lost to contact for ≥2 weeks despite repeated attempts (e.g., telephone, text message, home visit).

For lost-to-follow-up subjects, the investigator shall make every effort to complete the final safety assessment (e.g., vital signs, adverse event recording) and document the reason for withdrawal in the CRF.

(5) Provisions for Excluded Cases

A case is excluded from the efficacy analysis set if:

- The subject is misdiagnosed (e.g., final diagnosis is transient ischemic attack rather than ischemic stroke);
- The subject meets the inclusion criteria but is not administered any dose of hUC-MSC-sEV-001, or fails to receive scheduled doses due to production failure of the study drug;
- The subject participates in another interventional clinical trial during the study period;
- The subject receives prohibited combined medications (e.g., antineoplastic drugs, neurotrophic factors) or non-protocol-specified treatments that affect efficacy assessment;
- The subject has no evaluable efficacy data (e.g., no post-baseline NIHSS or mRS assessment);
- The subject has severe protocol violations (e.g., administration of the wrong dose, missed ≥3 doses).

Excluded cases shall be documented in the "Protocol Deviation Log", and their CRFs shall be retained for audit. Subjects who receive at least one dose of the study drug and have safety data shall be included in the safety analysis set.

(6) Criteria for Terminating or Withdrawing from the Study

i. Study Termination

Study termination refers to the early cessation of the entire study before completion of the planned protocol. The decision to terminate the study shall be made by the sponsor, principal investigator, or Ethics Committee, and shall be documented in the "Study Termination Report". Reasons for termination include:

- Emergence of SAEs that suggest a causal relationship with the study drug and pose a serious risk to subjects;
- Confirmation of the MTD or identification of an unsafe dose level;
- Major flaws in the study design that affect data validity;
- Regulatory requirements (e.g., request by the National Medical Products Administration [NMPA]).

. Subject Withdrawal

Subjects may withdraw from the study at any time for the following reasons:

(1) Investigator-determined withdrawal:

① The subject develops a medical condition that makes continued participation unsafe (e.g., severe allergic reaction, intracranial hemorrhage);② The subject uses medications that interact with the study drug (e.g., immunosuppressants) or affect efficacy assessment (e.g., edaravone);③ The subject experiences a SAE that requires termination of study participation;④ The subject has poor compliance that compromises the reliability of safety or efficacy data.

(2) Subject-initiated withdrawal:

① The subject voluntarily requests to withdraw (no reason required);② The subject is lost to follow-up (no contact for ≥2 weeks).

For withdrawn subjects, the investigator shall conduct a final safety assessment (if possible) and document the reason for withdrawal in the CRF. Subjects who withdraw due to AEs/SAEs shall receive appropriate medical treatment, and follow-up shall continue until the AE/SAE resolves or stabilizes.

(7) Criteria for Terminating the Study

The study will be terminated immediately if any of the following occurs:

≥2 subjects experience DLT at the same dose level (indicating that the dose exceeds the MTD);

- Production of hUC-MSC-sEV-001 fails to meet quality standards for 3 consecutive batches, resulting in inability to administer scheduled doses;

- The study drug is found to have no clinical benefit (e.g., no improvement in neurological function in all enrolled subjects) or is associated with unexpected severe toxicity;
- A major protocol deviation is identified (e.g., incorrect randomization, unapproved dose modification) that invalidates the study results;
- The sponsor terminates the study due to funding, strategic adjustments, or other reasons;
- The Ethics Committee or NMPA issues an order to terminate the study.

3. Intervention Measures

(1) Trial Drug

- **Generic Name**: Human Umbilical Cord Mesenchymal Stromal Cell-Derived Small Extracellular Vesicle (hUC-MSC-sEV-001) Nasal Drops
- **Source**: Isolated and purified from the supernatant of hUC-MSCs cultured under GMP conditions
- **Specification**: 2.0 mL/bottle; concentrations corresponding to the three dose groups: 1×10^{10} particles/mL, 5×10^{10} particles/mL, and 2.5×10^{11} particles/mL
- **Storage Conditions**: -80℃ ± 10℃ (ultra-low temperature refrigerator), protected from light; no repeated freezing-thawing
- **Pretreatment Agent**: Hyaluronidase for injection (150 U per nostril), used to improve nasal mucosa permeability
- **Administration Route**: Intranasal instillation (bilateral nostrils)

(2) Treatment Protocol

1. **Baseline Assessment**: Complete all screening assessments (laboratory tests, imaging, neurological scales) before the first dose.
2. **Nasal Mucosa Pretreatment**: Administer 150 U hyaluronidase (dissolved in 0.5 mL normal saline) to each nostril via a nasal dropper, and wait 15 minutes for absorption.
3. **Study Drug Administration**: Instill hUC-MSC-sEV-001 into bilateral nostrils using a dedicated dropper:
    - Low dose: 1 mL per nostril (total dose: 2×10^{10} particles);
    - Medium dose: 1 mL per nostril (total dose: 1×10^{11} particles);
    - High dose: 2 mL per nostril (total dose: 5×10^{11} particles).
4. **Administration Frequency**: Once daily for 4 consecutive days (Day 0: enrollment

day, Day 1, Day 2, Day 3).

5. **Concomitant Standard Treatment**: All subjects receive standard medical treatment for ischemic stroke in accordance with guidelines, including:
    - Antiplatelet therapy (e.g., aspirin 100 mg/day, clopidogrel 75 mg/day);
    - Antihypertensive therapy (target blood pressure: <140/90 mmHg for non-diabetic subjects, <130/80 mmHg for diabetic subjects);
    - Lipid-lowering therapy (target low-density lipoprotein cholesterol [LDL-C]: <1.8 mmol/L);
    - Glycemic control (target fasting blood glucose: 4.4-7.0 mmol/L for diabetic subjects).

(3) Rehabilitation Therapy

All subjects receive standardized rehabilitation therapy starting from Day 1 after enrollment, including:

- Manual therapy (e.g., joint mobilization, muscle strength training);
- Occupational therapy (e.g., activities of daily living training, fine motor skill training);
- Speech therapy (if applicable for subjects with aphasia);
- Physical therapy (e.g., gait training, balance training) as needed.

The type and duration of rehabilitation therapy are documented in the "Rehabilitation Therapy Record Form" in the CRF.

(4) Precautions During Intervention

1. **Dose Adjustment**: No dose reduction is allowed during the study. If a subject experiences a Grade ≥3 AE, study drug administration is suspended until the AE resolves to Grade ≤1; if the AE is judged to be related to the study drug, the subject withdraws from the study.
2. **Prohibited and Restricted Medications**:
    - Prohibited medications: Antineoplastic drugs, immunosuppressants (e.g., cyclosporine, tacrolimus), neurotrophic factors (e.g., nerve growth factor);
    - Restricted medications: Antibiotics and antiviral drugs (used only for confirmed infections, with duration documented); non-steroidal anti-inflammatory drugs (NSAIDs) (avoided if possible to reduce bleeding risk).

3. **Concurrent Diseases Management**: If a subject develops a concurrent disease (e.g., pneumonia, myocardial ischemia) during the study, the treating physician provides standard treatment, and the details are documented in the "Concurrent Disease Record Form" in the CRF.

4. **Subject Education**: Subjects are instructed to report any new symptoms (e.g., headache, nasal congestion, rash) to the investigator immediately, and to avoid self-medication with prohibited drugs.

4. Observation Items

(1) Demographic and Clinical Data

1. **Demographic Characteristics**: Gender, age, date of birth, ethnicity, dominant hand, occupation, height, weight, body mass index (BMI);

2. **Clinical History**: Chief complaint, onset time of stroke, detailed history of present illness, primary diagnosis (including infarct location), secondary diagnoses, past medical history (hypertension, diabetes, hyperlipidemia, coronary heart disease), surgical history, allergy history, family history, smoking history (pack-years), drinking history (alcohol consumption per week);

3. **Medication History**: Current medications (including over-the-counter drugs, herbal supplements) at screening, with dosage, frequency, and duration documented.

All data are recorded in the "Baseline Demographic and Clinical Data Form" in the CRF.

(2) Safety Observation Indicators

1. **Vital Signs**: Measured before each dose administration and at each follow-up visit:
    - Pulse (beats per minute), blood pressure (mmHg, measured in the sitting position after 5 minutes of rest), respiratory rate (breaths per minute), body temperature (℃, axillary or oral);
    - Abnormal vital signs (e.g., hypertension: systolic blood pressure ≥160 mmHg) are documented and followed up.

2. **Laboratory Tests**:
    - **Hematology**: Complete blood count (CBC) with differential (red blood cell [RBC], hemoglobin [Hb], white blood cell [WBC], neutrophil percentage [NEUT%], lymphocyte percentage [LYMPH%], platelet count [PLT]);
    - **Clinical Chemistry**: Liver function (ALT, AST, total bilirubin [TBIL], alkaline phosphatase [ALP], γ-glutamyl transferase [γ-GT]), renal function

(Scr, BUN), fasting blood glucose (FBG), glycated hemoglobin (HbA1c), lipid profile (total cholesterol [TC], triglycerides [TG], HDL, LDL, lipoprotein (a) [LP(a)]);

- **Coagulation Function**: PT, INR, APTT, thrombin time (TT), fibrinogen (FIB);
- **Serum Electrolytes**: Potassium (K^+), sodium (Na^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), phosphorus (P^{3+}), chlorine (Cl^-);
- **Infectious Disease Screening**: HBsAg, hepatitis B surface antibody (HBsAb), hepatitis B e antigen (HBeAg), hepatitis B e antibody (HBeAb), hepatitis B core antibody (HBcAb), HCV antibody, HIV antibody, TPPA, RPR;
- **Tumor Markers**: Alpha-fetoprotein (AFP), carbohydrate antigen 199 (CA199), carcinoembryonic antigen (CEA);
- **Urinalysis**: Urine protein (PRO), urine glucose (GLU), quantitative urine RBC, quantitative urine WBC, urine ketones (KET);
- **Pregnancy Test**: Urine pregnancy test for women of childbearing potential at screening and Day 90.

3. **Imaging Examinations**:
    - **Chest X-ray**: Performed at screening and Day 90 (to exclude active pulmonary infection or malignancy);
    - **Electrocardiogram (ECG)**: 12-lead ECG performed at screening, Day 14, and Day 90 (to evaluate cardiac function and detect arrhythmias);
    - **Cerebral Imaging**:
        - Baseline: NCCT, DWI, MRA/CTA (to confirm infarct location and vascular occlusion);
        - Day 14 and Day 90: DWI, fluid-attenuated inversion recovery (FLAIR), CTA/CT perfusion (CTP) (to assess infarct volume, edema, and cerebral perfusion).
4. **Adverse Events Monitoring**:

- AEs are monitored from the time of informed consent signing until 30 days after the last dose of the study drug.
- All AEs (including nasal irritation, headache, rash) are documented in the "Adverse

Event Record Form" in the CRF, with details including onset time, severity (CTCAE V5.0), duration, relationship to the study drug (definite, probable, possible, unlikely, unrelated), treatment measures, and outcome.

- SAEs are reported to the Ethics Committee, sponsor, and NMPA in accordance with regulatory requirements (within 24 hours of awareness).

(3) Efficacy Observation Indicators

1. **Neurological Function Scales**:
    - **NIHSS**: Assesses the severity of neurological deficits (range: 0-42; higher scores indicate more severe deficits). Administered at baseline, Day 7, Day 14, and Day 90.
    - **mRS**: Assesses functional independence (range: 0-6; scores 0-2 indicate good functional outcome). Administered at baseline, Day 7, Day 14, and Day 90.
    - **Modified Barthel Index (MBI)**: Assesses activities of daily living (range: 0-100; higher scores indicate better independence). Administered at baseline, Day 14, and Day 90.
    - **EQ-5D-5L**: Assesses health-related quality of life (including mobility, self-care, usual activities, pain/discomfort, anxiety/depression; scored as a utility value ranging from -0.291 to 1.0). Administered at baseline, Day 14, and Day 90.
2. **Cerebral Perfusion Parameters (CTP)**:

- Measured at baseline, Day 14, and Day 90: cerebral blood volume (CBV), cerebral blood flow (CBF), mean transit time (MTT), time to peak (TTP) in the infarct core and penumbra.
- Changes in perfusion parameters from baseline are used to evaluate the improvement of cerebral blood flow.

3. **Infarct Volume Measurement**:

- Infarct volume is measured on DWI using the ABC/2 method (A: maximum length, B: maximum width perpendicular to A, C: number of slices × slice thickness).
- Changes in infarct volume from baseline to Day 14 are used to evaluate infarct progression or regression.

5. Outcome Evaluation Indicators

(1) Primary Safety Endpoints

- **Dose-Limiting Toxicity (DLT)**: Assessed within 14 days of the first dose, defined as any of the following:

1. Grade 3-4 AE related to the study drug (CTCAE V5.0), such as severe nasal bleeding, anaphylactic reaction, or hepatic injury;

2. Increase in NIHSS score ≥ 4 points from baseline (indicating significant neurological deterioration);

3. Symptomatic intracranial hemorrhage (confirmed by CT/MRI and associated with neurological deterioration).

(2) Secondary Safety Endpoints

1. **SAE Incidence**: Proportion of subjects with drug-related SAEs within 90 days of the first dose;
2. **All-Cause Mortality**: Proportion of subjects who die from any cause within 90 days of the first dose;
3. **Laboratory Abnormality Rate**: Proportion of subjects with Grade ≥3 laboratory abnormalities (e.g., thrombocytopenia, elevated liver enzymes) within 90 days.

(3) Efficacy Endpoints

1. **Functional Outcome**: Proportion of subjects with mRS score 0-2 at Day 90;
2. **Neurological Improvement**: Mean change in NIHSS score from baseline to Day 14 and Day 90; proportion of subjects with NIHSS score reduction ≥4 points at Day 14;
3. **Functional Independence**: Mean change in MBI score from baseline to Day 90; proportion of subjects with MBI score ≥60 (indicating moderate independence) at Day 90;
4. **Quality of Life**: Mean change in EQ-5D-5L utility value from baseline to Day 90.

(4) Exploratory Endpoints

1. **Infarct Volume Change**: Mean change in infarct volume from baseline to Day 14;
2. **Cerebral Perfusion Improvement**: Mean changes in CBF, CBV, MTT, and TTP from baseline to Day 14;
3. **Correlation Analysis**: Correlation between baseline characteristics (e.g., age, NIHSS score) and efficacy outcomes.

6. Study Protocol Flow and Evaluation Indicators

First Visit [Baseline (Day 0)]

1. **Informed Consent**: The investigator explains the study protocol to the subject/legal representative, who signs the informed consent form if willing to participate.
2. **Screening Assessments**:
    - Demographic and clinical data collection;
    - Vital signs measurement;
    - Laboratory tests: CBC, clinical chemistry, coagulation function, serum electrolytes, infectious disease screening, tumor markers, urinalysis, pregnancy test (if applicable);
    - Imaging examinations: NCCT, DWI, MRA/CTA, chest X-ray, 12-lead ECG;
    - Neurological scales: NIHSS, mRS, MBI, EQ-5D-5L.
3. **Eligibility Confirmation**: The investigator reviews the inclusion/exclusion criteria and confirms eligibility.
4. **Study Drug Administration**:
    - Nasal mucosa pretreatment with hyaluronidase (150 U per nostril);
    - Instill the first dose of hUC-MSC-sEV-001;
    - Measure vital signs 30 minutes after administration.
5. **Documentation**: Record combined medications, rehabilitation therapy plans, and any AEs.

Second Visit (Day 7 ± 1)

1. **Safety Assessments**:
    - Vital signs measurement;
    - Laboratory tests: CBC, clinical chemistry, urinalysis;
    - AE monitoring and recording.
2. **Efficacy Assessments**:
    - Neurological scales: NIHSS, mRS.
3. **Documentation**: Record combined medications and rehabilitation therapy progress.

Third Visit (Day 14 ± 2)

1. **Safety Assessments**:
    - Vital signs measurement;
    - Laboratory tests: CBC, clinical chemistry, coagulation function, serum electrolytes, infectious disease screening, tumor markers, urinalysis;
    - Imaging examinations: 12-lead ECG, DWI, FLAIR, CTA/CTP;
    - AE monitoring and recording (including SAE follow-up).
2. **Efficacy Assessments**:
    - Neurological scales: NIHSS, mRS, MBI, EQ-5D-5L;
    - Infarct volume measurement (DWI) and CTP parameter analysis.
3. **Documentation**: Record combined medications, rehabilitation therapy, and schedule the Day 90 visit.

Fourth Visit (Day 90 ± 7)

1. **Safety Assessments**:
    - Vital signs measurement;
    - Laboratory tests: CBC, clinical chemistry, coagulation function, serum electrolytes, infectious disease screening, tumor markers, urinalysis, pregnancy test (if applicable);
    - Imaging examinations: Chest X-ray, 12-lead ECG, DWI, FLAIR, CTA/CTP;
    - Final AE follow-up (until 30 days after the last dose).
2. **Efficacy Assessments**:
    - Neurological scales: NIHSS, mRS, MBI, EQ-5D-5L;
    - Final infarct volume measurement and CTP parameter analysis.
3. **Study Completion**: Confirm completion of all assessments, and document any protocol deviations.

Items/Visits	Baseline (Day 0)	Day 7 (±1)	Day 14 (±2)	Day 90 (±7)
Sign Informed Consent Form	●			
Review Inclusion/Exclusion Criteria	●			
Collect Demographic & Clinical Data	●			
Vital Signs	●	●	●	●
Urine Pregnancy Test	●			●
Hematology (CBC) *	●	●	●	●
Clinical Chemistry (Liver/Kidney Function, Glucose, Lipids) *	●	●	●	●
Coagulation Function (PT, INR, APTT, FIB)	●		●	●
Serum Electrolytes	●		●	●
Infectious Disease Screening (8 Items)	●		●	●
Tumor Markers (AFP, CA199, CEA) *	●		●	●
Urinalysis *	●	●	●	●

Items/Visits	Baseline (Day 0)	Day 7 (±1)	Day 14 (±2)	Day 90 (±7)
Chest X-ray	●			●
12-Lead ECG	●		●	●
Cerebral Imaging (CT/MRI/CTA/CTP)	●		●	●
Neurological Scales (NIHSS, mRS)	●	●	●	●
Modified Barthel Index (MBI)	●		●	●
EQ-5D-5L Score	●		●	●
Record Adverse Events	●	●	●	●
Record Concomitant Medications	●	●	●	●
Record Rehabilitation Therapy	●	●	●	●

7. Regulations for Packaging, Acceptance, Storage, Distribution, and Recovery of Study MSC-sEV Preparations

(1) Packaging of Study MSC-sEV Preparations

- **Primary Packaging**: Sterile glass vials (2.0 mL) with rubber stoppers and aluminum caps, labeled with the study drug name, batch number, dose level, expiration date, storage conditions, and "For Clinical Trial Use Only".
- **Secondary Packaging**: Individual cartons containing one vial of study drug and one vial of hyaluronidase, with a leaflet describing the administration method.
- **Tertiary Packaging**: Insulated containers with dry ice for transportation, labeled with "Biological

Product", "Keep Frozen (-80°C)", and the sponsor's contact information.

After administration, empty vials and packaging materials are disposed of as medical waste in accordance with hospital regulations.

(2) Acceptance, Storage, and Distribution of Study MSC-sEV Preparations

1. Acceptance Procedures

- **Delivery Inspection**: Upon receipt of the study drug, the clinical research coordinator (CRC) checks the outer packaging for damage, verifies the temperature record (must be maintained at -80°C ± 10°C during transportation), and confirms the batch number, expiration date, and dose level match the delivery order.
- **Quality Inspection**: The pharmacist inspects the physical appearance of the study drug (clear, colorless liquid; no turbidity or precipitates) and verifies the certificate of analysis (CoA) provided by the manufacturer.
- **Documentation**: The CRC completes the "Study Drug Acceptance Form", attaches the temperature record and CoA, and stores them in the study file.

2. Storage Requirements

- **Storage Conditions**: The study drug is stored in a dedicated ultra-low temperature refrigerator (-80°C ± 10°C) with a temperature monitoring system that records temperature every 30 minutes. Alarms are triggered if the temperature exceeds the range.
- **Inventory Management**: A "Study Drug Inventory Log" is maintained, recording the batch number, dose level, quantity received, quantity administered, and remaining quantity. The refrigerator is locked, and access is restricted to authorized personnel (investigator, CRC, pharmacist).

3. Distribution Procedures

- **Drug Retrieval**: Before each dose administration, the CRC retrieves the study drug from the refrigerator and records the retrieval time in the inventory log.
- **Thawing**: The study drug is thawed at room temperature (20-25°C) for 15-20 minutes, and gently mixed before administration. No heating or shaking is allowed.
- **Administration Documentation**: After administration, the investigator documents the dose, administration time, and any deviations in the CRF and "Study Drug Administration Record Form".

(3) Recovery of Study MSC-sEV Preparations

- **Recoverable Scenarios**:

1. The study drug is expired, or the storage temperature exceeds the specified range;
2. The outer packaging is damaged, or the vial is cracked;

3. The study drug is contaminated (e.g., turbidity, precipitates);

4. The subject withdraws before receiving the scheduled dose;

5. The study is terminated early.

- **Recovery Procedures**:

1. The CRC labels the recoverable study drug with "To Be Recovered" and records the reason in the inventory log;

2. The study drug is returned to the sponsor in insulated containers with dry ice, accompanied by a "Study Drug Recovery Form";

3. The sponsor confirms receipt and issues a "Recovery Confirmation Certificate", which is stored in the study file.

8. Observation and Management of Adverse Events

(1) Definitions

- **Adverse Event (AE)**: Any untoward medical occurrence in a subject administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment. AEs include clinical signs (e.g., abnormal laboratory findings), symptoms, or diseases.
- **Serious Adverse Event (SAE)**: An AE that results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, causes persistent or significant disability/incapacity, or is a congenital anomaly/birth defect.
- **Adverse Drug Reaction (ADR)**: A response to a drug that is noxious and unintended, and which occurs at doses normally used in humans for the prophylaxis, diagnosis, or therapy of diseases, or for the modification of physiological function.
- **Dose-Limiting Toxicity (DLT)**: An AE that is severe enough to prevent dose escalation, as defined in Section 5.1 (Primary Safety Endpoints).

(2) Observation and Recording of Adverse Events

- **Monitoring Period**: From the time of informed consent signing until 30 days after the last dose of the study drug.
- **Reporting Requirements**: Subjects are instructed to report any new symptoms, signs, or discomfort to the investigator immediately. The investigator queries the subject about AEs at each visit using open-ended questions (e.g., "Have you experienced any new symptoms since your last visit?").
- **Documentation**: All AEs are recorded in the "Adverse Event Record Form" in the CRF, including:
    - Onset time, date, and duration;

- Severity (CTCAE V5.0: Grade 1 = mild, Grade 2 = moderate, Grade 3 = severe, Grade 4 = life-threatening, Grade 5 = death);
- Description of signs and symptoms (e.g., "nasal congestion, mild, lasting 2 days");
- Relationship to the study drug (assessed using the five-point scale: definite, probable, possible, unlikely, unrelated);
- Treatment measures (e.g., "normal saline nasal irrigation");
- Outcome (resolved, resolving, stable, worsening, fatal).

(3) Management of Adverse Events

- **Grade 1-2 AEs**: Monitor closely; provide symptomatic treatment if needed (e.g., antihistamines for mild rash). Continue study drug administration if the AE is judged to be unrelated or unlikely related to the study drug.
- **Grade 3-4 AEs**: Suspend study drug administration immediately; provide urgent medical treatment (e.g., corticosteroids for severe allergic reactions). The investigator assesses the causal relationship between the AE and the study drug. If the AE is related or probably related, the subject withdraws from the study, and follow-up continues until the AE resolves or stabilizes.
- **SAEs**: Implement the following emergency procedures:

1. Provide immediate medical treatment to stabilize the subject's condition;
2. Notify the principal investigator, sponsor, and Ethics Committee within 24 hours of awareness;
3. Complete the "Serious Adverse Event Report Form" and submit it to the Ethics Committee and sponsor;
4. Conduct follow-up assessments until the SAE resolves or stabilizes, and submit follow-up reports every 7 days until resolution.

(4) Assessment of Causal Relationship Between Adverse Events and Study Drug

The causal relationship is assessed by the investigator based on the following five factors:

1. **Temporal Relationship**: Whether the AE occurred after administration of the study drug (onset time within the drug exposure period);
2. **Known Pharmacological Effects**: Whether the AE is consistent with the known safety profile of MSC-sEVs (e.g., nasal irritation is a potential local effect);
3. **Dechallenge Response**: Whether the AE resolved or improved after discontinuing the study drug;
4. **Rechallenge Response**: Whether the AE recurred after re-administering the study drug (not applicable in this study due to safety concerns);

5. **Alternative Etiologies**: Whether the AE can be explained by the subject's underlying disease, concurrent medications, or other factors.

The causal relationship is classified into five categories:

- **Definite**: All five factors support a causal relationship;
- **Probable**: Four factors support a causal relationship, and no alternative etiology is identified;
- **Possible**: Three factors support a causal relationship, and alternative etiologies are possible;
- **Unlikely**: Two or fewer factors support a causal relationship, and alternative etiologies are likely;
- **Unrelated**: No factors support a causal relationship, and alternative etiologies are confirmed.

(5) Reporting of Serious Adverse Events

- **Reporting Timeline**: The investigator must report SAEs to the Ethics Committee, sponsor, and NMPA within 24 hours of becoming aware of the event.
- **Reporting Content**: The "Serious Adverse Event Report Form" includes the subject's initials, screening number, AE description, onset time, severity, causal relationship, treatment measures, outcome, and the investigator's signature.
- **Follow-Up Reporting**: If the SAE persists or worsens, follow-up reports are submitted every 7 days until the event resolves or stabilizes. A final report is submitted when the event resolves.

(6) Follow-Up of Adverse Events

- **Follow-Up Duration**: AEs are followed up until they resolve to Grade ≤1 or stabilize. For SAEs, follow-up continues until the event resolves or the subject's condition stabilizes.
- **Follow-Up Methods**: In-person visits, telephone calls, or video consultations, depending on the severity of the AE.
- **Documentation**: All follow-up information (e.g., changes in AE severity, treatment response) is recorded in the CRF and "Adverse Event Follow-Up Form".

9. Ethical Considerations

(1) Ethical Compliance in Protocol Design

- The study protocol is designed in accordance with the *Declaration of Helsinki (2022)*, *Good Clinical Practice (GCP)*, and the *Administrative Measures for Stem Cell Clinical Research (Trial)*.
- The protocol is reviewed and approved by the Ethics Committee of Zhujiang Hospital of Southern Medical University before study initiation. Any protocol amendments are submitted to the Ethics Committee for re-review and approval before implementation.

- The Ethics Committee monitors the study progress and may request modifications or termination if ethical concerns arise (e.g., unexpected severe toxicity).

(2) Informed Consent Process

- **Informed Consent Form (ICF)**: The ICF is written in plain language (avoiding medical jargon) and includes the following elements:
    - Purpose and duration of the study;
    - Study procedures (examinations, drug administration, follow-up);
    - Potential risks (e.g., nasal irritation, allergic reactions) and benefits (e.g., improvement in neurological function);
    - Alternative treatments (standard medical therapy, rehabilitation);
    - Right to withdraw at any time without penalty;
    - Compensation for SAEs (if the SAE is related to the study drug, the sponsor covers medical expenses and provides reasonable compensation);
    - Protection of privacy (data anonymization, restricted access to personal information).
- **Consent Process**: The investigator explains the ICF to the subject/legal representative in a private setting, answers questions, and ensures that the subject/representative fully understands the study before signing the ICF. A copy of the signed ICF is provided to the subject/representative.
- **Re-consent**: If the protocol is amended (e.g., new safety information emerges), the investigator re-explains the amended content to the subject/representative, who signs a new ICF if willing to continue participation.

(3) Protection of Subject Rights

- **Right to Withdraw**: Subjects may withdraw from the study at any time, for any reason, without affecting their access to standard medical care.
- **Right to Information**: Subjects are informed of new safety or efficacy data related to the study drug in a timely manner.
- **Compensation for Harm**: If a subject experiences a SAE related to the study drug, the sponsor covers all medical expenses incurred for treating the SAE and provides reasonable economic compensation in accordance with national regulations.

(4) Privacy and Data Protection

- **Data Anonymization**: Subject identifiers (name, ID number) are replaced with a unique screening number in the CRF and study database. The link between identifiers and screening numbers is stored in a

secure, password-protected file accessible only to authorized personnel.

- **Data Access**: Study data are accessible only to the investigator, CRC, monitor, statistician, and regulatory authorities. No personal identifiers are shared with third parties without the subject's consent.
- **Data Storage**: Paper records (ICF, CRF) are stored in a locked file cabinet in the study office; electronic data are stored on a secure server with encryption and regular backups. All data are retained for 30 years after study completion in accordance with GCP requirements.

(5) Ethical Oversight

- The Ethics Committee conducts regular reviews of the study (every 6 months) to assess subject safety, protocol compliance, and informed consent quality.
- The committee may conduct unannounced site visits to verify that the study is conducted in accordance with the approved protocol and ethical requirements.
- If the committee identifies ethical violations (e.g., coercion of subjects, falsification of data), it may issue a warning, suspend the study, or terminate the study.

10. Data Management

(1) Data Collection

- **Case Report Form (CRF)**: The CRF is designed using electronic data capture (EDC) software (e.g., Medidata Rave) with built-in logic checks to ensure data accuracy. The CRF includes modules for baseline data, safety data, efficacy data, and adverse events.
- **Data Entry**: The CRC enters data into the EDC system within 24 hours of each visit. The investigator reviews and approves the data within 48 hours to ensure accuracy and completeness.
- **Source Data Verification (SDV)**: The monitor verifies that data in the CRF match the source documents (medical records, laboratory reports, imaging films) to ensure data integrity. SDV is performed for 100% of subjects in this study.

(2) Data Validation

- **Logic Checks**: The EDC system performs real-time logic checks (e.g., "NIHSS score cannot be negative", "Day 14 visit cannot be scheduled before Day 7 visit") to identify data errors.
- **Query Generation**: For data inconsistencies or missing values, the data manager generates electronic queries in the EDC system, which are resolved by the investigator or CRC.
- **Query Resolution**: Queries must be resolved within 7 days of generation. The investigator provides a written explanation for each query, and the data manager verifies the resolution.

(3) Data Lock and Unlock

- **Data Lock**: The database is locked when all data are entered, all queries are resolved, and all subjects have completed the Day 90 visit. The lock process is documented in the "Data Lock Report", which is signed by the data manager, statistician, and sponsor.

- **Data Unlock**: If errors are identified after data lock, a formal data unlock request is submitted to the sponsor and data manager. The database is unlocked only if the errors affect the statistical analysis, and the unlock process is documented in the "Data Unlock Report".

(4) Data Quality Assurance

- **Training**: The CRC and investigator receive training on EDC system use, CRF completion, and data management procedures before study initiation.

- **Monitor Visits**: The monitor conducts regular site visits (every 4 weeks) to review data entry progress, resolve queries, and ensure compliance with data management procedures.

- **Audit**: The sponsor may conduct a data audit to verify data accuracy, SDV completion, and query resolution. The audit results are documented in the "Data Audit Report".

(5) Data Retention

- **Source Documents**: Medical records, laboratory reports, imaging films, and informed consent forms are stored in the hospital's medical record department or study office for 30 years after study completion.

- **Electronic Data**: The EDC database and backup files are stored on a secure server with restricted access. The data are retained for 30 years after study completion.

- **Statistical Analysis Data**: The locked database and statistical analysis code are stored in a secure location and retained for 30 years after study completion.

11. Statistical Analysis

(1) Statistical Analysis Sets

- **Full Analysis Set (FAS)**: Includes all randomized subjects who receive at least one dose of the study drug. Missing efficacy data are imputed using the last observation carried forward (LOCF) method.

- **Per Protocol Set (PPS)**: Includes subjects in the FAS who comply with the study protocol (e.g., complete all visits, receive all 4 doses of study drug, no major protocol violations). This set is used for sensitivity analysis of efficacy outcomes.

- **Safety Analysis Set (SAS)**: Includes all subjects who receive at least one dose of the study drug. This set is used for safety analyses (adverse events, laboratory abnormalities).

(2) Descriptive Statistics

- **Continuous Variables**: Presented as mean ± standard deviation (SD) for normally distributed data, or

median (P25, P75) for non-normally distributed data. Examples include age, NIHSS score, and infarct volume.

- **Categorical Variables**: Presented as frequency (percentage). Examples include gender, hypertension status, and mRS score categories (0-2 vs. 3-6).

(3) Inferential Statistics

- **Primary Efficacy Endpoint**: The proportion of subjects with mRS score 0-2 at Day 90 is compared between dose groups using the chi-square test or Fisher's exact test. A generalized linear model is used to adjust for potential confounders (e.g., age, baseline NIHSS score).
- **Secondary Efficacy Endpoints**:
    - Mean changes in NIHSS, MBI, and EQ-5D-5L scores from baseline are compared using repeated measures analysis of variance (ANOVA) or Kruskal-Wallis rank sum test.
    - The proportion of subjects with NIHSS score reduction ≥4 points at Day 14 is compared using the chi-square test.
- **Safety Endpoints**:
    - The incidence of AEs and SAEs is compared between dose groups using the chi-square test or Fisher's exact test.
    - Grade ≥3 laboratory abnormalities are summarized as frequency (percentage) by dose group.

(4) Sample Size Justification

This is a phase I dose-escalation study designed to explore the safety and MTD of hUC-MSC-sEV-001. Using the "3+3" design, a sample size of 6-18 subjects is sufficient to identify the MTD with a 90% probability of avoiding overdosing and a 80% probability of identifying the MTD if it exists.

(5) Statistical Software

All statistical analyses are performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). Two-sided tests are used, and a P-value < 0.05 is considered statistically significant.

(6) Interim Analysis

No interim analysis is planned for this study due to the small sample size. The study will be terminated early only if safety concerns arise (e.g., ≥2 subjects experience DLT at the same dose level).

12. Quality Control and Assurance

(1) Protocol Compliance

- **Training**: All study personnel (investigator, CRC, monitor, pharmacist) receive training on the study

protocol, GCP, and stem cell clinical research regulations before study initiation. Training records are retained in the study file.

- **Standard Operating Procedures (SOPs)**: SOPs are developed for key processes, including subject screening, study drug administration, adverse event reporting, and data management. Study personnel strictly follow the SOPs to ensure consistency.
- **Protocol Deviation Monitoring**: The monitor records all protocol deviations (e.g., missed visits, incorrect dose administration) in the "Protocol Deviation Log". Major deviations are reported to the Ethics Committee and sponsor.

(2) Study Drug Quality Control

- **Manufacturing Quality**: The study drug is produced under GMP conditions, and each batch is tested for purity, potency, sterility, and endotoxin levels. A certificate of analysis (CoA) is provided for each batch.
- **Storage and Transportation**: Temperature monitoring during transportation and storage ensures that the study drug remains within the specified temperature range (-80℃ ± 10℃). Temperature records are reviewed and archived.
- **Inventory Management**: The study drug inventory is checked monthly to ensure that the quantity administered matches the quantity received, and to prevent expiration or loss.

(3) Laboratory Quality Control

- **Laboratory Certification**: All laboratory tests are performed by a certified clinical laboratory (accredited by the College of American Pathologists [CAP] or the National Center for Clinical Laboratories [NCCL] of China).
- **Quality Control Samples**: The laboratory runs internal quality control samples (e.g., normal and abnormal controls for CBC) with each batch of tests to ensure accuracy. External quality assessment (EQA) is participated in to verify test reliability.
- **Reference Ranges**: Laboratory reference ranges are documented in the CRF and EDC system to ensure consistent interpretation of results.

(4) Monitoring and Audit

- **Monitor Visits**: The monitor conducts site visits every 4 weeks to review subject recruitment, data entry, SDV, and adverse event reporting. A monitoring report is submitted after each visit.
- **Audit**: The sponsor conducts a formal audit of the study site 6 months after study initiation to assess protocol compliance, data integrity, and ethical compliance. The audit results are documented in the "Audit Report".
- **Regulatory Inspection**: The NMPA may conduct an inspection of the study site to verify compliance

with GCP and regulatory requirements.

(5) Quality Assurance Statement

The principal investigator is responsible for the overall quality of the study. A quality assurance plan is developed to ensure that all study activities are conducted in accordance with the protocol, GCP, and regulatory requirements. Any quality issues are documented, investigated, and corrected in a timely manner.

13. Study Reporting

(1) Interim Reports

- Interim reports are submitted to the Ethics Committee and sponsor every 6 months, summarizing subject recruitment, adverse events, protocol deviations, and preliminary safety data.
- The interim report includes a safety overview (incidence of AEs/SAEs, DLT cases) and an update on study progress (number of subjects enrolled, number of subjects completing the Day 90 visit).

(2) Final Study Report

- The final study report is prepared by the principal investigator and statistician within 6 months of study completion. The report includes:
    - Executive summary;
    - Study background and objectives;
    - Study design and methods;
    - Subject demographics and baseline characteristics;
    - Safety results (AE/SAE incidence, DLT, laboratory abnormalities);
    - Efficacy results (functional outcomes, neurological improvement);
    - Exploratory results (infarct volume change, cerebral perfusion);
    - Discussion (interpretation of results, limitations, future directions);
    - Conclusions.
- The final report is reviewed and approved by the sponsor, and submitted to the Ethics Committee and NMPA for regulatory filing.

(3) Publication of Results

- The study results are submitted for publication in a peer-reviewed medical journal (e.g., *Stroke*, *Neurology*, *Cell Transplantation*) within 12 months of study completion.
- The publication follows the Consolidated Standards of Reporting Trials (CONSORT) guidelines for

reporting clinical trials, and includes a statement acknowledging the study sponsor and any conflicts of interest.

- All authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship, and the final manuscript is approved by all authors before submission.

14. Data Retention

(1) Retention Period

All study records (informed consent forms, CRFs, source documents, laboratory reports, imaging films, statistical analysis reports) are retained for 30 years after study completion in accordance with GCP requirements and national regulations.

(2) Retention Location

- **Paper Records**: Stored in a locked file cabinet in the Department of Neurosurgery and Oncology, Zhujiang Hospital of Southern Medical University, with restricted access to authorized personnel.
- **Electronic Records**: Stored on a secure server managed by the hospital's information technology (IT) department, with encryption, regular backups, and access controls (username and password).
- **Study Drug Records**: Records of study drug packaging, acceptance, storage, distribution, and recovery are retained in the hospital's pharmacy department.

(3) Access to Records

- During the retention period, authorized personnel (investigator, monitor, auditor, regulatory inspector) may access the records for study monitoring, audit, or regulatory inspection.
- Access to personal identifiers is restricted to the investigator and CRC, and requires written approval from the hospital's privacy officer.
- After the retention period, paper records are shredded in accordance with confidential waste disposal procedures, and electronic records are permanently deleted from the server.