

ORIGINAL STUDY PROTOCOL

Safety and feasibility of intranasal delivery of human dental follicle mesenchymal stem cel-derived exosomes for negative symptoms in treatment-resistant schizophrenia: A pilot study

Name of the ethic committee: Zigong Mental Health Center Ethics Committee

Approved No. of ethics committee: 20260101

Principal Investigators: Kezhi Liu (liukezhi@swmu.edu.cn)

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Study Summary

Title	Safety and feasibility of intranasal delivery of human dental follicle mesenchymal stem cel-derived exosomes for negative symptoms in treatment-resistant schizophrenia: A pilot study
Objective	<p>Primary Objective</p> <p>To assess the safety and feasibility of hDFSCs-Exo in the treatment of treatment-resistant schizophrenia.</p> <p>Secondary Objective</p> <p>To evaluate the preliminary efficacy of hDFSCs-Exo on negative symptoms of treatment-resistant schizophrenia.</p>
Participant	Treatment-resistant schizophrenia
Study Design	Single-center, open-label, single-arm, 3+3 dose-escalation design
hDFSCs-Exo	<p>Drug Name: Human Dental Follicle Mesenchymal Stem Cell-derived Exosomes (hDFSCs-Exo)</p> <p>Route of Administration: Intranasal spray</p> <p>Specification: 30 billion particles / 2 ml (particle concentration)</p> <p>Storage Conditions: Store at -80° C</p> <p>Manufacturer: Chengdu Shilian Kangjian Biotechnology Co.,</p>

	Ltd.
Dosage regimen	<p>This study plans to include five dose groups:</p> <p>1×10^9 particles/dose group</p> <p>2×10^9 particles/dose group</p> <p>4×10^9 particles/dose group</p> <p>8×10^9 particles/dose group</p> <p>1.6×10^{10} particles/dose group</p> <p>Nasal spray device: Disposable intranasal spray injector (Tianzhou Medical, model TZ-AN30)</p> <p>The exosomes will be prepared in 0.2 ml of normal saline and administered via intranasal spray twice weekly (with a 3-day interval between doses) for 8 weeks, totaling 16 administrations.</p> <p>Note: Prior to each treatment, nasal hygiene should be performed by irrigating both nasal cavities with a normal saline nasal spray. After waiting for 5 minutes, gently clean any secretions using a cotton swab.</p>
Inclusion criteria	<ol style="list-style-type: none"> 1. Diagnosed with schizophrenia according to ICD-10 criteria; 2. Aged between 18 and 60 years; 3. Long-term inpatients with a disease duration of more than 5 years; 4. No acute exacerbation in the past 6 months, and no change in medication regimen in the past 2 months; 5. Poor response to adequate dose and duration of treatment with at least two different antipsychotics, including clozapine; 6. Positive and Negative Syndrome Scale – Factor Score for Negative Symptoms (PANSS-FSNS) ≥ 24; 7. At least two of the three core negative symptom items on

	<p>the PANSS (N1, N4, and N6) scored ≥ 4;</p> <p>8. Clinical Global Impression – Severity of Illness (CGI-S) score ≥ 4;</p> <p>9. Signed written informed consent.</p>
Exclusion criteria	<p>1. History of severe allergic reactions;</p> <p>2. Definite organic brain lesions;</p> <p>3. Comorbid severe physical diseases (e.g., unstable coronary heart disease, malignant arrhythmia, hepatic or renal insufficiency, bronchial asthma, acute exacerbation of chronic obstructive pulmonary disease, autoimmune diseases, etc.);</p> <p>4. Current diagnosis of other mental disorders according to ICD-10 criteria (e.g., schizoaffective disorder, schizophreniform disorder, bipolar I disorder, bipolar II disorder, pervasive developmental disorder, mental retardation, delirium, dementia, amnestic disorder, or other cognitive disorders);</p> <p>5. Unstable condition requiring adjustment of medication regimen;</p> <p>6. Non-compliance with treatment;</p> <p>7. Severe rhinitis or nasal allergies;</p> <p>8. History of modified electroconvulsive therapy (MECT) within the past 3 months;</p> <p>9. Individuals at risk of suicide;</p> <p>10. Pregnant or lactating women;</p> <p>11. Other conditions deemed unsuitable for enrollment.</p>
Sample size	15-30

Measurement	<p>1. Primary Outcome Measures</p> <p>To assess the safety and feasibility of intranasal delivery of human dental follicle mesenchymal stem cell-derived exosomes.</p> <p>1) Safety Measures:</p> <p>(1) Adverse Events: Incidence and severity of adverse events (AEs) and serious adverse events (SAEs), graded according to CTCAE version 5.0.</p> <p>(2) Dose-Limiting Toxicity: Incidence of dose-limiting toxicities (DLTs), used to determine the maximum tolerated dose (MTD) and recommended Phase II dose (RP2D).</p> <p>(3) Vital Signs: Incidence of abnormalities in body temperature, pulse, respiration, and blood pressure.</p> <p>(4) Physical Examination: Incidence of abnormalities in general condition, head, skin, mucous membranes, lymph nodes, neck, chest, abdomen, spine/extremities, and neurological examination.</p> <p>(5) Laboratory Tests: Incidence of abnormalities in complete blood count, urinalysis, blood biochemistry (liver and kidney function), infectious disease markers, inflammatory factors, and allergy markers (IgE).</p> <p>(6) Electrocardiogram (ECG): Incidence of ECG abnormalities.</p> <p>2) Feasibility Measures:</p> <p>(1) Treatment Completion Rate: Proportion of subjects completing the full 8-week (16 sessions) intranasal</p>
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	<p>administration regimen.</p> <p>(2) Treatment Adherence: Evaluation of subjects' acceptance of and compliance with the intranasal administration method.</p> <p>2. Secondary Outcome Measures</p> <p>Changes from baseline to the end of Week 8 in the following measures:</p> <p>1) Change in Positive and Negative Syndrome Scale (PANSS) total score and factor scores.</p> <p>2) Change in Clinical Global Impression – Severity of Illness (CGI-S) score.</p> <p>3) Change in Scale for the Assessment of Negative Symptoms (SANS) total score and subscale scores.</p> <p>4) Change in Calgary Depression Scale for Schizophrenia (CDSS) score.</p> <p>5) Change in Montreal Cognitive Assessment (MoCA) total score.</p> <p>6) Change in Trail Making Test Part A/B (TMT-A/B) completion time.</p> <p>7) Change in Digit Span Test (DST) score.</p> <p>8) Change in Verbal Learning and Memory Test (VLMT) score.</p> <p>3. Exploratory Outcome Measures</p> <p>To explore changes in brain function from baseline to the end of Week 8, assessed by:</p> <p>1) Electroencephalography (EEG):</p>
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	<p>Changes in resting-state power spectrum in the prefrontal theta band (4–8 Hz).</p> <p>Changes in functional connectivity strength of the default mode network (DMN) based on source localization.</p> <p>2) Functional Near-Infrared Spectroscopy (fNIRS):</p> <p>Changes in oxygenated hemoglobin (Oxy-Hb) concentration in the bilateral dorsolateral prefrontal cortex (DLPFC) during a verbal fluency task.</p> <p>3) Magnetic Resonance Imaging (MRI):</p> <p>Changes in resting-state functional connectivity strength of the default mode network (DMN) and salience network (SN).</p>
Statistic analysis	<p>This study is a Phase I exploratory clinical trial. Statistical analyses will be primarily descriptive, with appropriate statistical inference methods applied for preliminary analyses of secondary and exploratory outcome measures. All statistical analyses will be performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). All statistical tests will be two-sided, and statistical significance will be set at $P < 0.05$. Given the exploratory nature of this study, no adjustment for multiple comparisons will be made for the results of secondary and exploratory outcome measures; findings should be interpreted as hypothesis-generating rather than confirmatory.</p> <p>All primary outcome measures, assessing safety and feasibility, will be analyzed using descriptive statistics only.</p>

	<p>Secondary outcome measures consist of changes from baseline to Week 8 in various scale scores. For continuous variables, normality will first be assessed using the Shapiro-Wilk test.</p> <p>If data are normally distributed, changes from baseline will be analyzed using the paired t-test, and the mean change with 95% confidence interval will be reported. If data are not normally distributed, changes from baseline will be analyzed using the Wilcoxon signed-rank test, and the median change with interquartile range will be reported.</p> <p>Changes in brain function parameters from baseline to Week 8 will be analyzed using the same statistical methods described above for continuous variables, with normality testing guiding the choice between parametric (paired t-test) and non-parametric (Wilcoxon signed-rank test) approaches.</p>
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1.Introduction

Schizophrenia is a severe chronic mental illness with high disability rate, affecting approximately 1% of the global population and imposing a substantial disease burden on patients, families, and society [1]. Schizophrenia is primarily characterized by positive symptoms (e.g., hallucinations, delusions, thought disorder, behavioral disturbances), negative symptoms (e.g., blunted affect, avolition, social withdrawal, alogia), and cognitive symptoms (e.g., memory impairment, executive dysfunction) [2]. The Treatment Response and Resistance in Psychosis (TRRIP) working group defines treatment-resistant schizophrenia (TRS) as persistent symptoms and functional impairment despite at least two adequate trials of antipsychotic medications [3], with approximately 25% of schizophrenia patients meeting TRS criteria [4]. Negative symptoms are considered a primary contributor to TRS [5] and a core factor leading to impaired social function and reduced quality of life [6]. Studies report that approximately 30%–60% of schizophrenia patients exhibit prominent negative symptoms [7]. Currently, schizophrenia treatment primarily relies on antipsychotics modulating dopamine and serotonin systems. However, both conventional and novel antipsychotics have shown limited efficacy in improving negative symptoms [8]. The pathophysiological mechanisms underlying negative symptoms are complex, involving interactions among prefrontal cortex and limbic system dysfunction, neuroinflammation, synaptic dysfunction, and neurotrophic factor deficiency [9]. This complexity renders medications targeting single neurotransmitter systems unlikely to achieve optimal efficacy.

Mesenchymal stem cells (MSCs) have garnered significant attention due to their potent anti-inflammatory, immunomodulatory, and neurotrophic properties [10]. Gobshtis N et al. demonstrated that a single intracerebroventricular injection of bone marrow-derived MSCs

successfully reversed schizophrenia-like behaviors and promoted hippocampal neurogenesis in a ketamine-induced schizophrenia mouse model [11]. Similarly, in an amphetamine-induced schizophrenia mouse model, a single intravenous injection of human umbilical cord-derived MSCs reversed schizophrenia-like behaviors and suppressed neuroinflammation [12]. Furthermore, studies have found that MSC-derived exosomes exhibit therapeutic effects comparable to those of MSCs, with advantages including low immunogenicity, reduced tumorigenic risk, enhanced ability to cross the blood-brain barrier, suitability for large-scale production and long-term storage, and improved safety profiles. These properties position MSC-derived exosomes as highly promising agents for treating central nervous system (CNS) disorders [13-15]. Zhong XL et al. reported that intranasal delivery of exosomes derived from olfactory ecto-mesenchymal stem cells restored synaptic plasticity and neurogenesis while reducing neuroinflammation in a methoxy methanol (MAM)-induced schizophrenia mouse model [16]. Similarly, in a phencyclidine (PCP)-induced schizophrenia mouse model, intranasal administration of MSC-derived exosomes rapidly accumulated in the prefrontal cortex, successfully reversed schizophrenia-like behaviors, and attenuated neuronal loss [17].

To our knowledge, although no clinical studies have yet reported the use of MSCs or their derived exosomes in schizophrenia patients, such approaches have been investigated in other neuropsychiatric disorders, including Alzheimer's disease [18, 19], Parkinson's disease [20], and autism [21]. For example, Professor Duk L. Na's team at Sungkyunkwan University School of Medicine in South Korea conducted a Phase I clinical trial involving intracerebroventricular injections (once monthly for three doses) of human umbilical cord blood-derived MSCs (hUCB-MSCs) in patients with mild-to-moderate Alzheimer's disease,

demonstrating the safety and feasibility of this approach [19]. Similarly, a recent Phase I clinical trial led by Professor Gang Wang's team at Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, investigated intranasal administration of human allogeneic adipose-derived MSC exosomes (twice weekly for 12 weeks) in mild-to-moderate Alzheimer's disease patients, finding good safety and potential cognitive decline mitigation [18]. Additionally, a Phase II randomized controlled trial revealed that three intravenous injections of MSCs ($10 \times 10^6/\text{kg}$) significantly improved motor function in patients with mild-to-moderate Parkinson's disease [20]. Given the substantial preclinical evidence supporting the antipsychotic efficacy of MSCs and their derived exosomes, it is reasonable to hypothesize that these interventions may also prove effective in schizophrenia patients.

Dental mesenchymal stem cells (DMSCs), originating from ectodermal neural crest cells, possess enhanced potential for neural lineage differentiation, promote neuronal axon elongation, exhibit low immunogenicity, and offer advantages in accessibility and reduced ethical concerns [22, 23]. Due to their unique origin and superior biological properties, DMSCs have emerged as "star cells" in regenerative medicine for neurological disorders, distinguishing themselves from other mesoderm-derived MSCs (e.g., bone marrow, adipose, umbilical cord) [24-26]. DMSCs demonstrate capacity for neural tissue regeneration and cerebral ganglion formation, expressing cellular markers and controlling growth factors that stimulate angiogenesis, thereby holding significant therapeutic potential in neurodegenerative diseases [27]. For instance, in a rat stroke model, Song M et al. compared the efficacy of human dental pulp MSCs (hDPSCs) and human bone marrow MSCs (hBMSCs), finding that both stem cell types effectively reduced infarct area compared to controls, with the hDPSC group showing significantly

greater improvement [28]. Furthermore, Pagella P et al. reported that hDPSCs expressed higher levels of neurotrophic factors (including NGF, BDNF, GDNF, and NT-3) than hBMSCs, and neurons co-cultured with hDPSCs in microfluidic systems developed longer axons than those co-cultured with hBMSCs [29]. In Alzheimer's disease animal models, DPSCs have also demonstrated anti-neuroinflammatory effects, mitochondrial repair, and cognitive improvement [30]. Additionally, Li F et al. reported that dental pulp stem cell-derived exosomes promoted axonal repair in spinal cord injury mice by inhibiting neuroinflammation and apoptosis [31]. Therefore, we hypothesize that dental-derived MSCs and their exosomes may offer superior therapeutic effects in CNS disorders compared to other MSC types. Dental follicle tissue, derived from unerupted tooth germs, offers the distinct advantage of abundant tissue availability, which is essential for obtaining sufficient cells to meet cell therapy pharmaceutical requirements. Previous research indicates that dental follicle stem cells (DFSCs) exhibit higher proliferation rates and clonogenic capacity compared to other dental-derived stem cells, suggesting that DFSCs may better satisfy clinical translation requirements in both quantity and quality [32]. Studies show that DFSCs display protein expression profiles more similar to cranial neural crest cells, suggesting potential advantages in treating neurological disorders [33]. Moreover, DFSCs demonstrate stronger inhibitory capacity against inflammatory cells and enhanced promotion of anti-inflammatory cells, indicating superior immunomodulatory capabilities [34].

Intranasal administration, as a non-invasive brain-targeted drug delivery route, is emerging as a novel strategy for treating CNS disorders. This pathway bypasses the blood-brain barrier, delivering drugs directly to the brain via olfactory and trigeminal nerve pathways, thereby enhancing brain bioavailability while reducing systemic side effects [35].

Preclinical studies have confirmed that intranasal administration of MSC-derived exosomes effectively improves neurological function in animal models of Alzheimer's disease [36], Parkinson's disease [37], stroke [38], and depression [39]. Additionally, intranasal delivery of olfactory ecto-mesenchymal stem cell-derived exosomes restored synaptic plasticity and neurogenesis while reducing neuroinflammation in schizophrenia mouse models [16]. Similarly, intranasal MSC-derived exosomes rapidly reached the prefrontal cortex, successfully reversed schizophrenia-like behaviors, and attenuated neuronal loss [17]. Tsivion H et al. suggest that intranasal delivery of MSC-derived exosomes offers advantages in feasibility and rapid access to brain lesion sites, potentially becoming a valuable future approach for treating schizophrenia, particularly negative symptoms [40]. However, no studies to date have reported intranasal delivery of dental-derived MSC exosomes for schizophrenia treatment.

Based on these findings, we hypothesize that intranasal delivery of human dental follicle mesenchymal stem cell-derived exosomes (hDFSCs-Exo) may target the brain via olfactory neural pathways, modulating neuroinflammation, promoting neurogenesis, and restoring synaptic plasticity, thereby ameliorating negative symptoms in treatment-resistant schizophrenia patients. Therefore, this study will be the first to explore the clinical application prospects of intranasal hDFSCs-Exo delivery for treatment-resistant schizophrenia, potentially offering a novel therapeutic strategy for this patient population.

2. Study Objectives

1) Primary Objective

To investigate the safety and feasibility of intranasal delivery of human dental follicle mesenchymal stem cell-derived exosomes (hDFSCs-Exo).

2) Secondary Objective

To explore the preliminary efficacy of intranasal delivery of hDFSCs-Exo in treating negative symptoms of treatment-resistant schizophrenia.

3. Study Subjects

3.1 Inclusion Criteria

- (1) Diagnosed with schizophrenia according to ICD-10 criteria;
- (2) Aged between 18 and 60 years;
- (3) Long-term inpatients with a disease duration of more than 5 years;
- (4) No acute exacerbation in the past 6 months, and no change in medication regimen in the past 2 months;
- (5) Poor response to adequate dose and duration of treatment with at least two different antipsychotics, including clozapine;
- (6) Positive and Negative Syndrome Scale – Factor Score for Negative Symptoms (PANSS-FSNS) ≥ 24 ;
- (7) At least two of the three core negative symptom items on the PANSS (N1, N4, and N6) scored ≥ 4 ;
- (8) Clinical Global Impression – Severity of Illness (CGI-S) score ≥ 4 ;
- (9) Signed written informed consent.

3.2 Exclusion Criteria

- (1) History of severe allergic reactions;
- (2) Definite organic brain lesions;
- (3) Comorbid severe physical diseases (e.g., unstable coronary heart disease, malignant arrhythmia, hepatic or renal insufficiency, bronchial asthma, acute exacerbation of chronic obstructive pulmonary disease, autoimmune diseases, etc.);
- (4) Current diagnosis of other mental disorders according to ICD-10 criteria (e.g., schizoaffective disorder, schizophreniform disorder, bipolar I disorder, bipolar II disorder, pervasive developmental

disorder, mental retardation, delirium, dementia, amnestic disorder, or other cognitive disorders);

- (5) Unstable condition requiring adjustment of medication regimen;
- (6) Non-compliance with treatment;
- (7) Severe rhinitis or nasal allergies;
- (8) History of modified electroconvulsive therapy (MECT) within the past 3 months;
- (9) Individuals at risk of suicide;
- (10) Pregnant or lactating women;
- (11) Other conditions deemed unsuitable for enrollment.

3.3 Withdrawal Criteria

- (1) Intolerance to the investigational treatment or occurrence of serious adverse reactions requiring discontinuation of treatment, in the judgment of the investigator;
- (2) Development of a serious physical illness requiring discontinuation of treatment, in the judgment of the investigator;
- (3) Request for discontinuation of treatment by the subject and/or their legal guardian;
- (4) Any other circumstances under which the investigator deems it necessary for the subject to withdraw from the study.

3.4 Termination Criteria

- (1) Occurrence of significant cluster serious adverse events during the trial that, in the investigator's judgment, necessitate study termination;
- (2) Identification of major errors in the clinical trial protocol that compromise the evaluation of drug effects, or occurrence of significant deviations during implementation of an otherwise well-designed protocol that, if continued, would impair the assessment of drug efficacy and safety;

- (3) Requirement for termination/suspension of the trial by the regulatory authority.

4. Study Design and Procedures

4.1 Study Design

This is a single-center, open-label, single-arm, 3+3 dose-escalation design comprising five dose groups. Dose escalation will be determined based on dose-limiting toxicities (DLTs). It is anticipated that 15–30 subjects will be enrolled.

4.2 Study Procedures

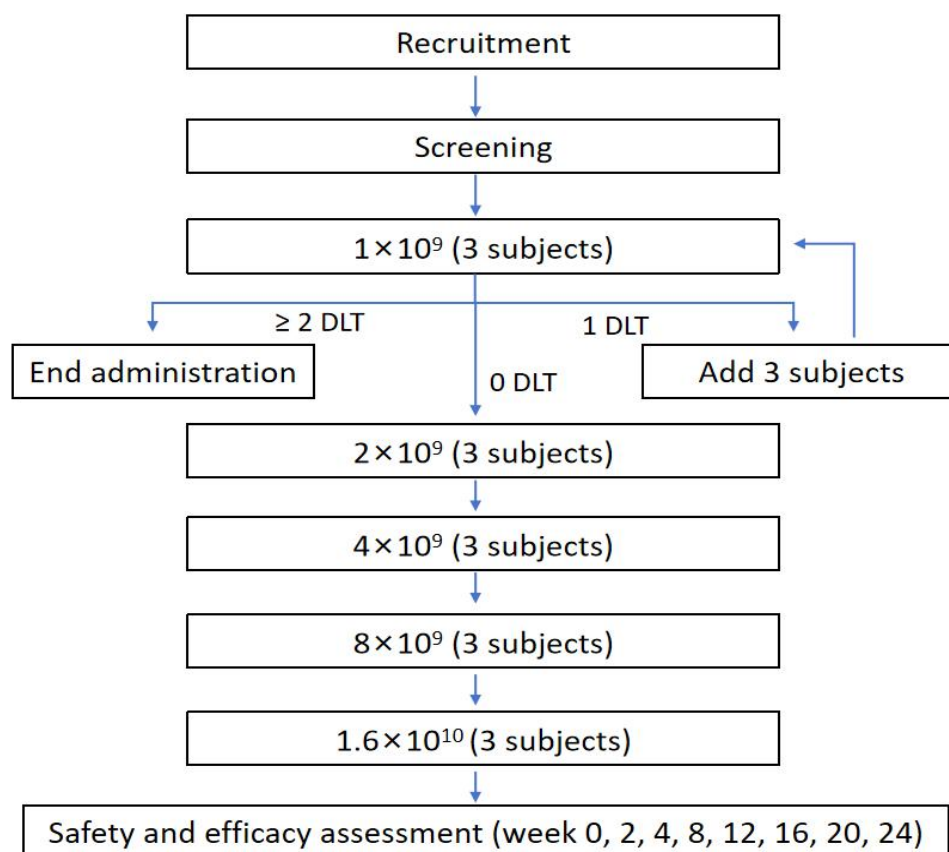
Five dose groups will be established, with dose escalation decisions guided by the occurrence of DLTs. DLTs will be assessed according to NCI-CTCAE version 5.0 criteria.

A DLT is defined as any toxicity possibly related or related to the investigational drug occurring during the DLT observation period (within 28 days after a single administration) that meets any of the following criteria:

- 1) Hematological Toxicity (lasting ≥ 7 days): Grade 4 neutropenia (absolute neutrophil count $< 0.5 \times 10^9/L$); Grade 3 thrombocytopenia with bleeding, or Grade 4 thrombocytopenia (platelet count $< 25 \times 10^9/L$); Grade 3 or Grade 4 anemia (hemoglobin < 8.0 g/dL).
- 2) Non-Hematological Toxicity: Any Grade 3 or Grade 4 non-hematological toxicity. Local Toxicity: Grade 3 or Grade 4 nasal mucosal inflammation, ulceration, or bleeding; Severe nasal congestion preventing nasal breathing. Systemic Allergic/Immune Reactions: Grade 3 or Grade 4 allergic reactions, systemic rash, or angioedema. Neurological Toxicity: New-onset, drug-related Grade 3 or Grade 4 headache, dizziness, sensory or motor nerve disorders. Systemic Inflammation: Grade 3 or Grade 4 fever (body temperature $> 39.0^\circ\text{C}$ and drug-related); Grade 3 or higher symptoms related to cytokine release

syndrome. Serious Adverse Events (SAEs): Any drug-related SAE, regardless of grade.

3) Laboratory Abnormalities: Grade 3 or Grade 4 hepatic or renal function abnormalities (e.g., ALT/AST elevation $> 5 \times$ upper limit of normal, creatinine elevation $> 3 \times$ upper limit of normal), judged by the investigator to be related to the investigational drug.



5. Research Methods

5.1 Exosome Preparation and Characterization

Clinical-grade human dental follicle mesenchymal stem cell-derived exosomes (hDFSCs-Exo) will be provided by Chengdu Shilian Kangjian Biotechnology Co., Ltd. (specification: 30 billion particles/2 mL). Each batch has a shelf life of 30 days. The exosomes will be transported via dry ice refrigeration and stored at -80°C upon arrival at the hospital. Before use, they will be thawed at room temperature and gently shaken to

ensure uniformity. Exosome morphology will be observed using transmission electron microscopy (TEM). Particle size distribution and concentration will be determined using nanoparticle tracking analysis (NTA). Marker protein expression will be detected via Western blotting, including positive markers CD9, CD63, CD81, Alix, and TSG101, and negative markers GM130 and Calnexin.

5.2 Intervention Protocol

1) Dose Determination:

The intranasal dose for exosome treatment in schizophrenia mouse models was reported as 2.4×10^7 particles per administration [17]. Based on body surface area conversion (0.007 m^2 for a 20 g mouse and 1.53 m^2 for a 60 kg adult), the equivalent human dose was calculated as 5.54×10^9 particles per administration. Applying a 5-fold safety factor, the starting dose was reduced to 1.1×10^9 particles per administration. This was further considered alongside the safe dose of 8×10^8 particles per administration used in intranasal exosome treatment for Alzheimer's disease patients [18]. To explore the maximum tolerated dose (MTD) and recommended Phase II dose (RP2D), five dose groups are planned: 1×10^9 , 2×10^9 , 4×10^9 , 8×10^9 , and 1.6×10^{10} particles per administration.

2) Administration Method:

Exosomes will be dissolved in 0.2 mL of normal saline and administered via intranasal spray, alternating between both nostrils. Treatment will be administered twice weekly (with a 3-day interval between doses) for 8 weeks, totaling 16 administrations. Nasal hygiene will be performed prior to each treatment session.

3) Exosome Skin Test:

To avoid potential allergic reactions, subjects will undergo a wrist skin test prior to the first treatment. The skin test will be performed by a

clinical nurse, with results assessed 20 minutes after administration. Results will be classified as negative (no reaction at the test site; no rash with itching, no papules, no surrounding urticaria, redness, or swelling) or positive (presence of marked rash, papules, or urticaria; surrounding congestion, swelling, blisters, itching, etc.) [38].

5.3 Assessment Content and Schedule

1) Safety and Feasibility Assessments:

- (1) Vital signs monitoring
- (2) Electrocardiogram (ECG)
- (3) Complete blood count
- (4) Liver and kidney function tests
- (5) Allergy markers (IgE)
- (6) Inflammatory markers
- (7) Recording of adverse events (AEs) and serious adverse events (SAEs)
- (8) Treatment completion rate

2) Efficacy Assessments:

- (1) Positive and Negative Syndrome Scale (PANSS) negative subscale score
- (2) Scale for the Assessment of Negative Symptoms (SANS) score
- (3) Calgary Depression Scale for Schizophrenia (CDSS) score
- (4) Montreal Cognitive Assessment (MoCA) score
- (5) Clinical Global Impression (CGI) scale score
- (6) Trail Making Test Parts A & B
- (7) Digit Span Test
- (8) Verbal Learning and Memory Test

3) Other Assessment Tools:

- (1) Electroencephalography (EEG)
- (2) Functional near-infrared spectroscopy (fNIRS)
- (3) Functional magnetic resonance imaging (fMRI)

4) Assessment Schedule

	Screen -ing	Base -line	Intervention			Follow-up			
Visiting Week	V1 -2	V2 0	V3 2	V4 4	V5 8	V6 12	V7 16	V8 20	V9 24
Informed consent	X								
Enrollment	X								
Demographic data	X								
Medical history	X								
Physical examination		X	X	X	X	X	X	X	X
Vital signs		X	X	X	X	X	X	X	X
Lab examination		X	X	X	X	X	X	X	X
PANSS	X			X	X	X			X
SANS		X		X	X	X			X
CDSS		X		X	X	X			X
CGI	X			X	X	X			X
MoCA		X		X	X	X			X
TMT-A		X		X	X	X			X
TMT-B		X		X	X	X			X
DST		X		X	X	X			X
VLMT		X		X	X	X			X
Inflammatory factor		X		X	X	X	X	X	X
ECG		X	X	X	X	X	X	X	X
EEG		X			X				
fNIRS		X			X				
MRI		X			X				
AEs & SAEs		X	X	X	X	X	X	X	X

6. Outcome Measures

6.1 Primary Outcome Measures

1) Safety Measures:

(1) Adverse Events: Incidence and severity of adverse events (AEs) and serious adverse events (SAEs), graded according to CTCAE version 5.0.

(2) Dose-Limiting Toxicity: Incidence of dose-limiting toxicities (DLTs), used to determine the maximum tolerated dose (MTD) and recommended Phase II dose (RP2D).

(3) Vital Signs: Incidence of abnormalities in body temperature, pulse, respiration, and blood pressure.

(4) Physical Examination: Incidence of abnormalities in general condition, head, skin, mucous membranes, lymph nodes, neck, chest, abdomen, spine/extremities, and neurological examination.

(5) Laboratory Tests: Incidence of abnormalities in complete blood count, urinalysis, blood biochemistry (liver and kidney function), infectious disease markers, inflammatory factors, and allergy markers (IgE).

(6) Electrocardiogram (ECG): Incidence of ECG abnormalities.

2) Feasibility Measures:

(1) Treatment Completion Rate: Proportion of subjects completing the full 8-week (16 sessions) intranasal administration regimen.

(2) Treatment Adherence: Evaluation of subjects' acceptance of and compliance with the intranasal administration method.

6.2 Secondary Outcome Measures

(1) Change in Positive and Negative Syndrome Scale (PANSS) total score and factor scores.

(2) Change in Clinical Global Impression --Severity of Illness (CGI-S) score.

(3) Change in Scale for the Assessment of Negative Symptoms (SANS) total score and subscale scores.

(4) Change in Calgary Depression Scale for Schizophrenia (CDSS) score.

(5) Change in Montreal Cognitive Assessment (MoCA) total score.

(6) Change in Trail Making Test Part A/B (TMT-A/B) completion time.

(7) Change in Digit Span Test (DST) score.

(8) Change in Verbal Learning and Memory Test (VLMT) score.

6.3 Exploratory Outcome Measures

(1) Electroencephalography (EEG): Changes in resting-state power spectrum in the prefrontal theta band (4 - 8 Hz), and changes in

functional connectivity strength of the default mode network based on source localization.

(2) Functional Near-Infrared Spectroscopy (fNIRS): Changes in oxygenated hemoglobin (Oxy-Hb) concentration in the bilateral dorsolateral prefrontal cortex (DLPFC) during a verbal fluency task.

(3) Magnetic Resonance Imaging (MRI): Changes in resting-state functional connectivity strength of the default mode network (DMN) and salience network (SN).

7. Statistic analysis

This study is a Phase I exploratory clinical trial. Statistical analyses will be primarily descriptive, with appropriate statistical inference methods applied for preliminary analyses of secondary and exploratory outcome measures. All statistical analyses will be performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). All statistical tests will be two-sided, with statistical significance set at $P < 0.05$. Given the exploratory nature of this study, no adjustment for multiple comparisons will be made for the results of secondary and exploratory outcome measures; findings should be interpreted as hypothesis-generating rather than confirmatory.

Primary outcome measures (safety and feasibility) will be analyzed using descriptive statistics exclusively. For secondary outcome measures, which assess changes from baseline to Week 8 in various scale scores, the following approach will be applied to continuous variables: normality will first be assessed using the Shapiro-Wilk test. If data are normally distributed, changes from baseline will be analyzed using the paired t-test, with the mean change and 95% confidence interval reported. If data are not normally distributed, changes from baseline will be analyzed using the Wilcoxon signed-rank test, with the median change and interquartile

range reported. Exploratory outcome measures (changes in brain function parameters assessed by EEG, fNIRS, and MRI) will be analyzed using the same statistical methods described above for continuous variables, with normality testing guiding the selection between parametric (paired t-test) and non-parametric (Wilcoxon signed-rank test) approaches.

8 .References

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