Source and Number of the Project：

Randomized Controlled Study of Mesenchymal Stem Cell Therapy for Chronic Kidney Disease

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Implementing Unit: The First Affiliated Hospital of Air Force Medical University

Study Duration: 2024-01-01 – 2027-08-31

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PROTOCOL SYNOPSIS

Study Title	Randomized Controlled Study of Intravenous Mesenchymal Stem Cell Injection for Chronic Kidney Disease
Objective	To investigate the therapeutic effect of allogeneic umbilical cord tissue-derived mesenchymal stem cell intravenous injection therapy on chronic kidney disease.
Design	This trial is a prospective, randomized, controlled clinical trial. Eligible subjects will be randomized in a 1:1 ratio to either the experimental group or the control group. The experimental group will receive a single intravenous injection of allogeneic umbilical cord tissue-derived MSCs（mesenchymal stem cells） at a dose of $1.0*10^6$/kg. Baseline data will be collected before treatment. Follow-up assessments will be conducted at 1, 3, 6, and 12 months post-treatment to evaluate the efficacy for CKD(chronic kidney disease) patients (Stage 3 and 4).
Sample Size	32 subjects
Subject Selection	Inclusion Criteria: (1) Agreement to participate in the trial and provision of signed written informed consent; (2) Pathological diagnosis of diabetic nephropathy or hypertensive renal damage; (3) 15 ≤ eGFR(estimated glomerular filtration rate) ＜ 60 mL/min/1.73m², UACR(urinary albumin-to-creatinine ratio) > 300 mg/g; (4) Age ≥ 18 years.
	Exclusion Criteria: (1) Extremely severe anemia (hemoglobin < 30 g/L);

	(2) Received blood product transfusion therapy within 1 month; (3) Autosomal dominant or recessive polycystic kidney disease (ADPKD); (4) History of kidney transplant or other solid organ transplant; (5) Active systemic or localized infection (e.g., pneumonia, osteomyelitis); (6) Allergy to stem cells themselves or stem cell-related culture medium; (7) History of allergic reaction to cell products (e.g., blood transfusion, platelets); (8) History of coagulation disorders (thromboembolism, pulmonary embolism, deep vein thrombosis); (9) History of malignancy or current malignant disease; (10) Elevated tumor markers (AFP, CEA, CA199, CA125, etc.); (11) Pregnant women or women with plans for pregnancy within 3 months after MSC（mesenchymal stem cell）therapy; (12) Participation in drug-related clinical trials within the past 2 months; (13) Any form of drug abuse, mental illness, or other conditions considered by the investigator as potentially affecting the trial's validity or the subject's health.
Intervention	Eligible subjects will be randomized 1:1 to the experimental group or the control group. The experimental group will receive a single intravenous injection of allogeneic umbilical cord tissue-derived MSCs at a dose of $1.0*10^6$/kg. The control group

	will receive a placebo. Baseline data will be collected pre-treatment. Follow-ups at 1, 3, 6, and 12 months post-treatment will guide subjects to return to the hospital for relevant laboratory tests. The primary endpoints are estimated glomerular filtration rate (eGFR) and urinary albumin-to-creatinine ratio (UACR). Secondary endpoints include other renal function parameters, other organ system function parameters, and MSC-related adverse events. The MSCs for treatment will be provided by Shaanxi Zhonggang Wanhai Institute of Life Sciences Co., Ltd. or Shaanxi Yisaier Biotechnology Co., Ltd.
Endpoints	Efficacy Endpoints: Changes in eGFR, 24-hour urine protein, and UACR at 1, 3, 6, and 12 months will serve as primary endpoints to assess renal function improvement. Changes in other renal function parameters (e.g., quantitative urine protein, urine red blood cells, serum creatinine, cystatin C),and changes in other organ system function parameters (e.g., ECG/echocardiography, complete blood count, liver function, NT-proBNP, parathyroid hormone, 25-dihydroxyvitamin D, lymphocyte subsets) at 1, 3, 6, and 12 months will serve as secondary endpoints.
	Safety Endpoints: Incidence of adverse events.
Statistical Methods	An EDC database will be established. All data and results will be entered into the database. Statistical analysis will be performed using SPSS software, including t-tests, Wilcoxon rank-sum tests, chi-square tests, Spearman correlation analysis,and Cox regression analysis, etc.
Study Period	January 2024 – August 2027

1. Background

Chronic Kidney Disease (CKD) refers to chronic structural and functional abnormalities of the kidneys caused by various etiologies, encompassing a group of diseases with different causes, pathologies, and varying severities[1]. CKD is defined as the presence of kidney damage markers or a glomerular filtration rate (GFR) below 60mL/(min·1.73m²) for more than 3 months. Besides GFR, the severity of proteinuria is also an important factor influencing the progression rate and outcomes of CKD[2]. The top three etiologies of CKD in Chinese patients are primary glomerulonephritis, diabetic nephropathy, and hypertensive nephrosclerosis. CKD is a progressive, incurable disease with high morbidity and mortality, posing a serious public health problem worldwide. Its global average prevalence is 9.1%, affecting approximately 697.5 million patients, with nearly 132 million in China[3]. Current treatment strategies include therapies to delay the progression of chronic renal insufficiency and treatments for various complications, namely general treatment, treatment of the primary disease, and measures to slow the progression of chronic renal failure. When the disease progresses to end-stage renal disease (ESRD), patients can only undergo renal replacement therapy, including hemodialysis, peritoneal dialysis, and kidney transplantation[4].

Mesenchymal Stem Cells (MSCs) are multipotent adult stem cells with self-renewal capacity, derived from the mesoderm. They have become a focus of advanced biomedical technology worldwide and have demonstrated safety and efficacy in clinical trials for various diseases[5], even being included in expert consensus for liver disease treatment[6]. MSCs have the potential to promote regeneration of damaged renal tissue, achieving renal protection and even reversal of injury, offering a new possibility and supplement to existing therapies.

Currently, clinical trials of MSCs for kidney diseases have mainly focused on lupus nephritis[7, 8], diabetic nephropathy[9-11], and acute kidney injury[12]. Their safety has been consistently verified, but due to generally small sample sizes and a lack of long-term follow-up, their efficacy remains controversial[13]. Therefore, our research team plans to conduct this randomized controlled clinical trial. Through long-term follow-up and observation of changes in multiple renal and other organ function parameters, we aim to further validate the therapeutic effect of MSCs on patients with severe chronic kidney disease (CKD stages 3 and 4), providing new ideas and methods for the clinical treatment and prognosis improvement of CKD patients.

2. Study Objectives

To evaluate the effect of intravenous injection of umbilical cord tissue-derived mesenchymal stem cells on the improvement of renal function in patients with chronic kidney disease (CKD stages 3 and 4), using the change in estimated glomerular filtration rate (eGFR) as the primary outcome measure, and changes in other renal function laboratory

parameters, changes in other organ system function laboratory parameters, and the incidence of adverse events as secondary outcomes. This trial aims to further verify and evaluate the therapeutic effect of MSCs on patients with severe chronic kidney disease (CKD stages 3 and 4), providing new ideas and strategies for expanding clinical treatment options, delaying progression, and improving the prognosis of CKD patients.

3. Study Design

This study is a prospective, randomized controlled clinical trial initiated by the team of Associate Professor He Lijie from the Nephrology Department of Xijing Hospital. Eligible subjects will be randomized in a 1:1 ratio to the experimental group or the control group. The experimental group will receive a single intravenous injection of allogeneic umbilical cord tissue-derived MSCs at a dose of $1.0*10^6$/kg. The control group will receive a placebo. Baseline data will be collected before treatment. Follow-up assessments will be conducted at 1, 3, 6, and 12 months post-treatment, guiding subjects to return to the hospital for relevant laboratory tests. The primary endpoints are the estimated glomerular filtration rate (eGFR) and urinary albumin-to-creatinine ratio (UACR) at 1, 3, 6, and 12 months after treatment. Secondary endpoints include other renal function parameters, other organ system function parameters, and MSC-related adverse events. The MSCs for treatment are prepared by Shaanxi Zhonggang Wanhai Institute of Life Sciences Co., Ltd. or Shaanxi Yisaier Biotechnology Co., Ltd.

4. Subject Selection

4.1 Inclusion Criteria

(1) Agreement to participate in the trial and provision of signed,

written informed consent;

(2) Pathological diagnosis of diabetic nephropathy or hypertensive renal damage;

(3) 15≤eGFR＜60min·1.73m²，UACR＞300mg/g；

(4) Age ≥ 18 years.

4.2 Exclusion Criteria

(1) Extremely severe anemia (hemoglobin < 30 g/L);

(2) Received blood product transfusion therapy within 1 month;

(3) Autosomal dominant or recessive polycystic kidney disease (ADPKD);

(4) History of kidney transplant or other solid organ transplant;

(5) Active systemic or specific organ infection (e.g., pneumonia, osteomyelitis);

(6) Known hypersensitivity to mesenchymal stem cells or any component of the cell culture medium;

(7) History of severe allergic or anaphylactic reactions to biological products (e.g., blood-derived products);

(8) History of coagulation disorders (thromboembolism, pulmonary embolism, deep vein thrombosis);

(9) History of malignancy or current malignant disease;

(10) Elevated tumor markers (AFP, CEA, CA199, CA125, etc.);

(11) Pregnant women or women planning pregnancy within 3 months after MSC therapy;

(12) Participation in drug-related clinical trials within the past 2 months;

(13) Any form of drug abuse, mental illness, or other conditions considered by the investigator as potentially affecting the trial's validity or the subject's health.

4.3 Study Termination Criteria

Criteria for subject withdrawal include:

(1) Occurrence of serious adverse events or complications related to MSC injection or the CKD disease itself;

(2) Loss to follow-up or death of the subject;

(3) Voluntary request for withdrawal by the subject or their legal guardian.

The withdrawal rate will be calculated at the end of the study. The reason and date of withdrawal will be recorded in detail. For subjects who withdraw from the study, appropriate treatment will be provided based on the judgment of the investigators at the participating center, and follow-up of their treatment outcomes may continue.

5. Methods and Technical Route

5.1 Interventions

All eligible patients will be randomly assigned to the experimental group (MSC group) or the control group (placebo group), while continuing to receive conventional therapy. The experimental group will receive an intravenous injection of allogeneic MSCs at a dose of

1.0*10^6/kg. The control group will receive a placebo, which is an intravenous infusion of 250 mL of 0.9% sodium chloride injection, matching the appearance and administration procedure of the MSC infusion..

5.2 Sample Size Determination

This trial is an exploratory study. A total of 32 subjects are planned to be enrolled, with 16 each for hypertensive nephropathy and diabetic nephropathy, as a preliminary exploration of the treatment. For each pathological type, subjects will be randomly allocated in a 1:1 ratio to the experimental and control groups, meaning 8 subjects per group per pathology (total experimental group n=16, control group n=16).

5.3 Randomization

Subjects who provide informed consent will be allocated in a 1:1 ratio to the experimental group or the control group. Computer-generated randomization will be used to determine group assignment. A random number sequence will be generated using SPSS 27.0 (or higher) software (provided by a statistical professional). According to this sequence, corresponding group codes will be placed into sealed, opaque envelopes. Upon sequential enrollment of subjects, the group assignment will be determined by the code inside the envelope, administering the corresponding treatment.

5.4 Data Collection and Follow-up

5.4.1 Information Collected

Table 1. Study Visit Schedule and Data Collection

Study Visit	creening/Baseline/Randomization	Post-treatment Month 1, 3, 6, 12	Month 1,3,6,12 and End of Study
Sign Informed Consent	√		
Medical History	√	√	√
Symptoms and Signs	√	√	√
ECG or Echocardiography (if needed)	√	√	√
Routine Examinations	√	√	√
Treatment Modality	√	√	√
Concomitant Medication Record	√	√	√

Renal Function Related Parameters	√	√	√
Primary Endpoint Events	√	√	√
Secondary Endpoint Events	√	√	√

Note: All adverse events and side effects of the drug will be recorded. Concomitant medications will be recorded and categorized by drug type.

5.4.2 Baseline Data

(1) Demographic information: age, gender, ethnicity, BMI, blood pressure, smoking history, clinical diagnosis, etc.

(2) Laboratory tests:

* Renal and Liver Function: eGFR, serum creatinine, cystatin C, blood urea nitrogen, serum albumin, cholesterol, triglycerides, LDL, HDL, uric acid, blood glucose.

* Other System Functions: NT-proBNP, PTH, 1,25-(OH)2-Vitamin D3, complement, T lymphocytes.

* Urinalysis: protein qualitative, urine red blood cell count.

* 24-hour urine protein quantification (mg/24h).

* Complete Blood Count: red blood cell count, hemoglobin.

* Tumor Markers (AFP, CEA, CA125, CA199, etc.).

(3) Baseline treatment regimen: use of antihypertensive, lipid-lowering,

hemostatic, and other relevant medications.

5.4.3 Follow-up Data

(1) Primary and secondary endpoints;

(2) Routine laboratory tests:

* Renal and Liver Function: eGFR, serum creatinine, cystatin C, blood urea nitrogen, serum albumin, cholesterol, triglycerides, LDL, HDL, uric acid, blood glucose.

* Other System Functions: NT-proBNP, PTH, 1,25-(OH)2-Vitamin D3, complement, T lymphocytes.

* Urinalysis: protein qualitative, urine red blood cell count.

* 24-hour urine protein quantification (mg/24h).

* Complete Blood Count: red blood cell count, hemoglobin, etc.

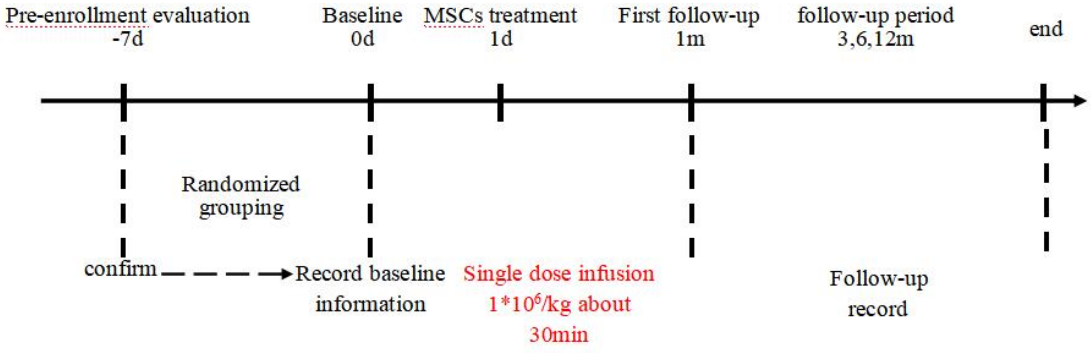
(3) Imaging examinations: ECG, echocardiography, renal/renal vessel ultrasound, chest CT, etc. (if necessary).

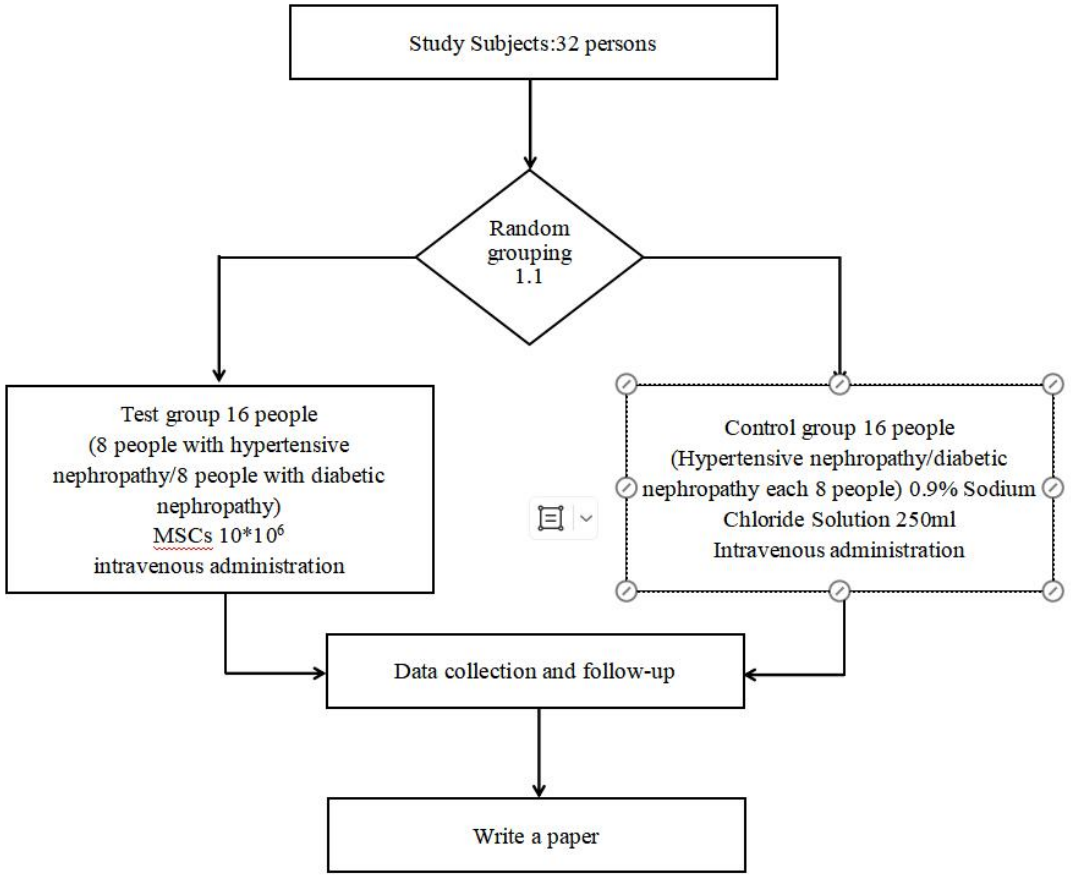
5.4.4 Strategies to Ensure Data Integrity

(1) Routine Clinical Visit Data Collection: This study will use paper Case Report Forms (CRFs) for data collection. An electronic data capture (EDC) system (or an Access database) will be used for data management. Double data entry with comparison will be employed for data entry and verification, followed by corresponding data checks and cleaning. During the study period, the principal researchers will review the trial data monthly to ensure internal consistency.

(2) Subject Reminders: Automated reminder messages will be sent to subjects in advance via WeChat group, SMS, or phone calls to minimize sample loss due to loss to follow-up and/or withdrawal.

5.5 Technical Route Diagram





6. Observation Items and Time Points

Primary Endpoints: The estimated glomerular filtration rate (eGFR) and 24-hour urine protein quantification levels at 1, 3, 6, and 12 months will serve as the primary outcome measures.

Secondary Endpoints: Changes in other renal function parameters (urine red blood cells, cystatin C, blood urea nitrogen, urine protein/creatinine ratio, etc.), changes in other organ system function parameters (ECG/echocardiography, complete blood count, liver function, NT-proBNP, parathyroid hormone, 25-dihydroxyvitamin D, etc.), immune-inflammatory markers (interleukins, CRP, T-cell subset analysis, complement levels, etc.), and the incidence of adverse events at 1, 3, 6, and 12 months will serve as secondary outcomes.

Severity will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL).

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death related to adverse event.

7. Observation of Adverse Events

Potential adverse reactions associated with MSC therapy may include: (1) Systemic immunogenic reactions to the injected cells, local inflammation, and long-term immune responses; (2) Cell dysfunction, viral infection, and post-injection infectious complications; (3) Fever, local pain; (4) Pulmonary and renal thromboembolism, cardiac and hepatic fibrosis; (5) although the mainstream view suggests that MSC therapy may inhibit tumor occurrence and metastasis, there are reports indicating a potential increased risk of tumor recurrence; (6) Xenogeneic contamination reactions potentially leading to acute inflammatory responses, etc.

8. Quality Control and Quality Assurance

The investigators will be responsible for screening subjects according to the inclusion/exclusion criteria, recording research data in the CRFs, and entering the data into the electronic database. Simultaneously, they will strictly adhere to the timeline and maintain close contact with the subjects to avoid enrolling ineligible subjects, prevent subjects from voluntarily withdrawing, ensure subjects receive the assigned treatment, and prevent crossover between groups. A Data and Safety Monitoring Board (DSMB) composed of two statisticians or clinicians experienced in clinical research design and data analysis will be established. The DSMB will

independently monitor the study, oversee randomization, data management, safety monitoring, and statistical analysis, blinded to the specific group assignments and treatments of the subjects. Once quality assurance procedures are completed, the database will be locked.

9. Statistical Analysis

All statistical analyses will be performed based on the Intention-to-Treat (ITT) principle, regardless of poor compliance, withdrawal, or loss to follow-up. We will conduct independent observational analyses of the two treatment regimens and report the trial results accurately. Continuous variables conforming to a normal distribution will be expressed as mean ± standard deviation, and comparisons between two groups will use T-tests, while comparisons among multiple groups will use analysis of variance (ANOVA). Non-normally distributed continuous variables will be expressed as median and interquartile range [M (P25, P75)]. Categorical variables will be expressed as percentages, and comparisons between groups will use chi-square tests. Results will be reported as Hazard Ratios (HR) with 95% Confidence Intervals (CI). The decline rate of eGFR will be compared between groups using a linear mixed-effects model. Survival analysis will employ Cox regression models. Considering the exploratory nature and purpose of this preliminary study, the limited sample size, and the consequent relatively limited statistical power, we will conduct objective preliminary comparative analyses and

interpret the results cautiously. All statistical analyses will be performed using SPSS 27.0 or R version 4.2.3 software.

10. Ethics of the Clinical Research

The clinical research will adhere to the World Medical Association's Declaration of Helsinki and other relevant regulations. Before the study begins, the protocol must be approved by the Institutional Review Board/Ethics Committee. Prior to enrolling any subject in this study, the investigator is responsible for providing the subject or their legal representative with a complete and comprehensive explanation of the study's purpose, procedures, potential benefits, and risks. Written informed consent must be obtained. Subjects should understand that they have the right to withdraw from the study at any time. The informed consent forms will be retained as clinical research documents for future reference. The privacy of subjects and the confidentiality of their data will be protected throughout the research process.

11. Study Timeline

Jan. 1, 2024 – Jan. 31, 2025: Preliminary preparation, study protocol design, investigator meeting, ethics committee review, trial registration.

Feb. 1, 2025 – Jul. 31, 2026: Trial initiation, patient enrollment and follow-up, monitoring visits, data collection, management of blood samples and adverse drug events.

Aug. 1, 2026 – Aug. 31, 2027: Data consolidation, document archiving, statistical analysis, manuscript writing and publication, reporting of results.

12. References

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