Informed Consent Form

Dear patient:

We would like to invite you to participate in a clinical study of "Safety and Efficacy of Umbilical Cord Mesenchymal Stem Cell Exosomes in the Treatment of Chronic Cough after COVID-19 Infection". This research plan has been reviewed and approved by the Ethics Committee of Wuhan Union Hospital, Huazhong University of Science and Technology.

Before you decide whether to participate in this study, please read the following information carefully. It will help you understand the study, why it is being conducted, the procedures and timeline of the study, the benefits, risks, and discomfort that may result from participating in the study. If you wish, you can also discuss with your family or friends or ask your doctor for further explanation to help you decide.

I. Introduction

1. Research Title and Researchers

The name of this project is "Safety and Efficacy of Umbilical Cord Mesenchymal Stem Cell Exosomes in the Treatment of Chronic Cough after COVID-19 Infection". The research unit is Wuhan Union Hospital, Huazhong University of Science and Technology, and the project's principal investigator is Dr. Jihui Du.

2. Background

2.1 Symptoms and Treatment Strategies of Post-COVID-19 Syndrome (Long COVID)

COVID-19 has been raging globally for more than three years, causing more than 5 million deaths. According to the World Health Organization (WHO), about 10%-20% of COVID-19 patients experience persistent symptoms after acute COVID-19 infection. Common symptoms include fatigue, shortness of breath, persistent (chronic) cough, loss of smell or taste, muscle pain, memory, and attention problems ("brain fog"), etc. These symptoms usually persist for at least 2 months, cannot be explained by other diagnoses, and occur at least 3 months after COVID-19 infection, which is called "post-COVID-19 syndrome" or "long COVID".

Research shows that over-activated immune cells in the lungs produce many cytokines through a positive feedback cycle, forming an inflammatory storm, which is the main pathological mechanism of COVID-19 pneumonia. After COVID-19 infection, the proportion of post-COVID cough is about 18%. This is mainly due to the invasion of the respiratory mucosa by the virus, leading to local inflammatory reactions and airway hyperresponsiveness. Moreover, COVID-19 is neurotropic and can easily cause

neurological damage, making cough more severe and persistent than that caused by common cold or flu.

The medical strategy for treating COVID-19 mainly involves using antiviral drugs to block the replication cycle of the virus and various means to suppress excessive inflammation to improve symptoms. Currently, commonly used anti-inflammatory and immunotherapy methods in clinical practice mainly include steroids, monoclonal antibody drugs, etc. These are mainly aimed at more severe inflammatory reactions and have issues such as low bioavailability and significant side effects. Existing therapies still have various limitations, and overall efficacy needs to be improved.

2.2 Mechanisms of Umbilical Cord Mesenchymal Stem Cell-Derived Exosomes Treatment

Umbilical cord mesenchymal stem cells (MSCs) have been demonstrated to possess comprehensive and powerful immune regulation and regeneration functions. MSCs can combat cell death associated with the pathogenesis of chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis, asthma, acute respiratory distress syndrome (ARDS) and pulmonary arterial hypertension and promote cellular regeneration. **Exosomes are one of the key paracrine effectors secreted by MSCs** and, due to their biomolecular similarity to parental cells, **possess immunomodulatory and regenerative repair capabilities**. In comparison to stem cells, **exosomes have no potential risk, low immunogenicity, high stability, and are easy to store**. Therefore, they are considered as promising candidates for replacing MSCs in treating various diseases.

According to the latest version of the "Diagnosis and Treatment Protocol for Novel Coronavirus Infection (Trial Tenth Edition)" published by the National Health Commission, **the main medical strategies for treating COVID-19 include supportive care, antiviral therapy, and immune therapy**. Immune therapy aims to inhibit excessive inflammatory responses and reduce damage to lung tissue. Developing new low-side-effect and cost-effective immune regulation methods is of great significance in treating post-COVID-19 syndrome. Previous studies have shown that exosomes secreted by MSCs can be used to treat other respiratory diseases such as immune deficiencies, inflammation, ARDS, acute lung injury, and COPD. MSC-derived exosomes can regulate immunity through interactions with immune cells and suppress inflammation through cytokine inhibition. Therefore, MSC-derived exosomes may be an effective method for treating long COVID.

Numerous clinical trials at home and abroad have shown that intravenous infusion of MSCs and MSC-EVs is a safe and effective treatment for severe lung damage caused by SARS-CoV-2. The size of MSC exosomes is between 30-150 nm, and after atomization, they can directly reach the bronchioles and alveoli, facilitating

maximal drug absorption. In the case of lung injury, atomized inhalation is a particularly effective route of administration. Therefore, we speculate that atomized inhalation of MSC exosomes may be an effective method for treating COVID-19 post-infection syndrome.

2.3 Clinical cases of extracellular vesicle therapy

In 2021, **Direct Biologics' ExoFloTM injection was approved by the US FDA for the treatment of COVID-19 in phase I/II clinical trials**. ExoFloTM reduces sustained inflammation and promotes blood vessel regeneration in damaged tissues. No reportable adverse events were observed in the phase II clinical trial. Recently, **Exo-CD24, an inhaled anti-COVID exosome developed by Israeli medical center expert Nadir Arber**, showed significant efficacy. In a phase I clinical trial involving 30 severe patients, 29 were cured after using it for 5 days.

China has also actively launched related clinical research. **Shanghai Ruijin Hospital and Jinyintan Hospital jointly conducted HAMSCs-Exos extracellular vesicle atomized treatment for COVID-19 in 2020**. No adverse events were found in all 7 severe patients who underwent atomized inhalation of exosomes. After atomized inhalation, all patients' pulmonary lesions improved to varying degrees, with 4 cases showing significant improvements. Preliminary research results show that participants' pulmonary injuries have been significantly improved, and no adverse events were observed. T**he clinical study on umbilical cord mesenchymal stem cell extracellular vesicle atomization treatment for COVID-19 conducted by the Fifth People's Hospital of Wuxi City, Jiangsu Province** was published in the journal Stem Cell Reviews and Reports in June this year. 7 patients diagnosed with COVID-19 pneumonia (including 2 severe cases and 5 mild cases) received treatment without acute or secondary allergic reactions, and no adverse events were reported. Chest CT scans after treatment showed that exosomes significantly promoted the absorption of pulmonary lesions and shortened hospital stays for severe and mild patients. **These studies demonstrate the feasibility of umbilical cord mesenchymal stem cell extracellular vesicle atomization therapy for COVID-19**.

3. Research objective

This study aims to evaluate the safety and effectiveness of umbilical cord mesenchymal stem cell exosomes (atomized inhalation) in the treatment of chronic cough after COVID-19 infection through a non-randomized controlled clinical trial.

4. Study design and grouping

This study adopts a non-randomized controlled clinical trial design. The study will be conducted at the Wuhan Union Hospital of Huazhong University of Science and

Technology, with a sample size of 80 cases, according to a 1:1 ratio (40 cases in the experimental group and 0 cases in the control group). If you agree to participate, you will receive treatment with umbilical cord mesenchymal stem cell exosomes (experimental group) through atomized inhalation based on the routine treatment plan.
(1) Experimental group: umbilical cord mesenchymal stem cell extracellular vesicle preparation; specification: 5ml, with an extracellular vesicle concentration of 1*10^9 particles/ml.
(2) Control group: patients who have not received extracellular vesicle atomized inhalation treatment during the same period.

5. Inclusion criteria

(1) The patient voluntarily agrees to participate in this study and signs an informed consent form.
(2) The age of the subject at the time of signing the informed consent form should be ≥18 years or ≤80 years, males and females are not limited.
(3) The subject was previously diagnosed with COVID-19 (positive nucleic acid or antigen detection) with symptoms lasting for more than 4 weeks.
(4) The subject is negative for nucleic acid or antigen at the time of screening.
(5) The subject has had persistent or intermittent cough or olfactory and taste disorders for ≥4 weeks without similar symptoms before the COVID-19 infection.
(6) The subject has not undergone umbilical cord mesenchymal stem cell extracellular vesicle-related treatment.
(7) The patient fully understands the purpose and requirements of this trial and is willing to complete all trial procedures according to the trial requirements.

6. Participant's research participation time and duration, follow-up frequency and process

The project cycle is 28 days, including 5 stages: screening/baseline period (day 0), treatment period (days 1-5), efficacy evaluation period 1 (day 6), efficacy evaluation period 2 (day 14), and follow-up period (day 28).

7. Contents that require participant's cooperation

(1) You should comply with the requirements of the researchers and relevant staff.
(2) If you withdraw from the study halfway, you should try to find your doctor for final evaluation.

II. If participating in the study, what will participants need to do?

1. Before you are selected for the study, the doctor will ask about and record your

medical history and screen you according to the inclusion and exclusion criteria and perform relevant examinations during the screening period. If you meet the criteria, you can voluntarily participate in the study and sign an informed consent form. If you do not wish to participate in the study, we will treat you according to your wishes.
2. If you voluntarily participate in the study, the following steps will be taken:

2.1 Visit 1 (Screening/baseline period, day 0)

The doctor evaluates whether the patient meets the inclusion criteria and introduces the relevant information about the study to the patient and signs an informed consent form. The study staff needs to inquire about the patient's medical history, complete the post-acute sequelae of SARS-CoV-2 infection symptom questionnaire survey and relevant laboratory tests (complete blood count, CRP, liver, and kidney function), or pulmonary CT, pulmonary function tests on day 0. If the patient meets all the inclusion criteria and does not meet all the exclusion criteria, he or she will be randomized into a group and receive the corresponding treatment plan according to the random grouping result.

2.2 Treatment period (days 1-5)

According to the treatment plan and grouping, start nebulization treatment for patients from day 1 (skin test should be performed before nebulization on day 1). The treatment cycle is 5 days, with 2 doses per day. The nebulization time is more than 10 minutes until the nebulized preparation is used up. Record the patient's temperature, blood pressure, heart rate, respiratory rate, and other changes before or 30 minutes after nebulization treatment and record adverse events.

2.3 Visit 2 (Efficacy evaluation period 1, phone follow-up, day 6)

After the completion of treatment, on the 6th day after the patient's inclusion in the study, a telephone follow-up is conducted according to relevant requirements, and the trial participant completes a symptom questionnaire survey on the 6th day, recording adverse events.

2.4 Visit 3 (Efficacy evaluation period 2, day 14)

On the 14th day after the start of the trial, corresponding examinations are conducted according to relevant requirements: (1) Vital signs, including temperature, blood pressure, heart rate, and respiratory rate; (2) Laboratory tests, including complete blood count, blood biochemistry (liver and kidney function), and serum inflammatory markers (CRP); (3) Pulmonary CT or pulmonary function tests. Participants are required to complete symptom questionnaires to assess the relief of cough and other symptoms and record adverse events.

Participants who complete all the items of this visit will complete the trial and enter the follow-up period.

2.5 Follow-up period (day 28, phone follow-up)

Safety and efficacy follow-up is conducted on the 28th day after inclusion, and the trial participant is required to complete a symptom questionnaire survey, record the results of the patient questionnaire survey, and collect adverse events.

2.6 Unscheduled visit

During the trial, for the safety of the trial participant, if an adverse event or abnormal laboratory test occurs, the researcher may increase the number of follow-up visits for the participant as needed, namely unscheduled visits. The researcher must accurately record each unscheduled visit of the participant in the relevant data, such as the original medical records and case report forms.

III. Possible benefits of participating in the study

Participating in this study may or may not improve your health condition.

The potential benefits of participating in this study for patients include that nebulized inhalation of mesenchymal stem cell-derived exosomes may have a certain alleviating effect on chronic cough and loss of taste and smell caused by long COVID-19 symptoms and may promote the improvement of relevant symptoms and shorten the recovery time. The information obtained from this study may have a favorable impact on the treatment of post-COVID-19 sequelae in the future. Although there is evidence that mesenchymal stem cell-derived exosomes have satisfactory efficacy in COVID-19 patients, this does not guarantee its efficacy for you. The nebulized inhalation of mesenchymal stem cell-derived exosomes used in this study is not the only method for treating post-COVID-19 sequelae.

IV. Participating in the study of potential adverse reactions, risks, discomfort, inconvenience, and related damages

During the study, you may experience adverse reactions, including but not limited to: allergic reactions, mild fever, diarrhea, or shortness of breath. We will monitor any adverse reactions of all participants in the study. If you experience any adverse reactions during the study, please contact the researcher immediately. You can also tell your family or close friends that you are participating in a clinical study so they can be aware of the events described above. If they have any questions about your participation in

the study, you can tell them how to contact the researcher.

If your health is damaged due to your participation in this study, please notify the researcher immediately, and they will be responsible for taking appropriate treatment measures and compensating you according to relevant regulations. The researcher will not compensate for damages caused by failure to follow the study procedures.

V. Treatment-related expenses and insurance expenses

5.1 Treatment-related expenses

To compensate you for any inconvenience caused by your participation in this study, the study will provide stem cell-derived extracellular vesicle drugs required for the study free of charge, pay a transportation allowance (200 yuan) and a nutrition allowance (300 yuan) during your participation in the study, and provide one blood routine, inflammation markers (CRP), liver and kidney function tests, and lung CT or pulmonary function tests (determined by the doctor according to your condition) and consultation fee on the 14th day after inclusion. Routine clinical diagnosis and treatment costs unrelated to this study will be borne by you.

5.2 Insurance expenses

If you experience adverse reactions, physical injury, or illness during the study, please inform your research doctor immediately. The doctor will provide medical treatment and clinical treatment advice and try to prevent any damage caused by this study. If you have any problems during the study, you can contact the doctor in charge of the study at any time.

The study has purchased clinical trial insurance. If any adverse events related to the study treatment or process occur, the research institution will provide reimbursement and compensation for the treatment costs according to relevant laws and regulations.

VI. Confidentiality of personal information

Your medical records (research medical records/CRF, laboratory results, etc.) will be kept intact at the hospital where you received treatment. The doctor will record the laboratory and other examination results in your medical record. The researcher, ethics committee, and scientific research management department will be allowed to access your medical records. Any public report on the results of this study will not disclose your personal identity. Within the limits allowed by law, we will make every effort to protect the privacy of your personal medical information.

According to medical research ethics, except for personal privacy information, the trial data will be available for public inquiry and sharing, limited only to web-based

electronic databases, ensuring that no personal privacy information is leaked.

VII. How to get more information?

You can ask any questions about this study at any time and receive corresponding answers. If there is any important new information during the study that may affect your willingness to continue to participate, your doctor will notify you promptly.

VIII. Participation in the study and withdrawal from the study are voluntary

Whether to participate in the study depends entirely on your own will. You can refuse to participate in this study or withdraw from the study at any time during the study, which will not affect your relationship with the doctor nor cause any other losses or benefits.

For your best interests, the doctor or researcher may suspend your continued participation in this study at any time during the study.

If you withdraw from the study for any reason, you may be asked about your participation in the clinical study. If deemed necessary by the doctor, you may also be required to undergo laboratory tests and physical examinations.

IX. What should you do now?

Whether to participate in this study is your (and your family's) decision.

Before deciding to participate in the study, please ask your doctor as many questions as possible.

If you have any questions after signing up, you can consult the department responsible physician of the Clinical and Translational Medicine Center: Name: Du Jihui, Contact: 26553111-30218, or the contact person of the Ethics Committee: Huang Xiaojia, Contact Number: 26553111-25509.

Thank you for reading the above materials. If you decide to participate in this study, please inform your doctor, who will arrange all matters related to the study for you. Please keep this information.

Informed Consent Form-signature Page

Clinical research project title: Safety and efficacy of umbilical cord mesenchymal stem cell-derived exosomes for treating chronic cough after COVID-19 infection

Sponsor: Huazhong University of Science and Technology Union Shenzhen Hospital.

Consent statement:

I have read the information provided regarding this study and have had the opportunity to discuss it with a physician and ask any questions I may have had. All my questions have been satisfactorily answered.

I understand the potential risks and benefits of participating in this study. I also confirm that my participation is voluntary, and I have had sufficient time to consider my decision. I am aware that:

- I can consult with the physician at any time to obtain more information.
- I can withdraw from the study at any time without fear of discrimination or retaliation, and my medical treatment and rights will not be affected.

If I terminate my participation during the study, specifically due to drug-related issues, I will inform the physician of any changes in my condition and complete any necessary physical and chemical examinations, which will be beneficial to the entire study.

If I require any other medication for my changing condition, I will seek the physician's advice beforehand or inform the physician truthfully afterwards.

I agree that the management department for scientific research, ethical committees, or project team members may access my research data. I will receive a signed and dated copy of the informed consent form.

In conclusion, I have decided to participate in this study and promise to follow medical advice to the best of my ability.

Your Signature: Date:

Phone Number:

I confirm that I have explained the detailed information regarding this trial to the patient, including their rights, potential benefits, and risks. I have also provided them with a signed copy of the informed consent form.

Doctor’s Signature: Date:

Phone Number: