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Allogeneic human umbilical cord-derived mesenchymal stem/stromal cells for chronic obstructive pulmonary disease (COPD): study protocol for a matched case-control, phase I/II trial.

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Abstract

Introduction

The global prevalence of chronic obstructive pulmonary disease (COPD) is increasing, and it has become a major public health burden worldwide, including in Vietnam. A large body of preclinical and clinical studies supports the safety and efficacy of mesenchymal stem/stomal cells (MSCs) in the treatment of lung injury, including COPD. The aim of this trial is to investigate the safety and potential therapeutic efficacy of allogeneic administration of umbilical cord-derived MSCs (UC-MSCs) as a supplementary intervention in combination with standard COPD medication treatments in patients with moderate-to-severe COPD based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2019 and Vietnam Ministry of Health's guidelines.

Methods and analysis

This matched case-control phase I/II trial is conducted at Vinmec Times City International Hospital, Hanoi, Vietnam between June 2020 and December 2021. In this study, 40 patients will be enrolled and assigned into two age-, gender- and COPD condition-matched groups, including a UC-MSC group and a control group. Both groups will receive standard COPD medication treatment based on the GOLD 2019 guidelines and the Vietnam Ministry of Health protocol. The UC-MSC group will receive two doses of thawed UC-MSC product with an intervention interval of 3 months. The primary outcome measures will include the incidence of prespecified administration-associated adverse events (AEs) and serious adverse events (SAEs). The efficacy will be evaluated based on the absolute changes in the number of admissions, arterial blood gas analysis, lung function and lung fibrosis via CT scan and chest X-ray. The clinical evaluation will be conducted at baseline and 3, 6, and 12 months post intervention.

Ethics and dissemination

Ethical approval was secured from the Ethical Committee of Vinmec International Hospital (number: 166/2019/QĐ-VMEC) and Vietnam Ministry of Health (number: 2002/QĐ-BYT).

Trial registration number: NCT04433104.

Strengths and Limitations

- This project is the first matched case-control phase I/II study to evaluate the safety and efficacy of allogeneic administration of UC-MSCs as supplementary treatment in combination with standard medication treatments for patients with moderate-to-severe COPD.
- To address the challenge of evaluating the effectiveness of MSC treatment in COPD by using quantitative and qualitative research methods.
- To interlink the treatment effectiveness with stem cell phenotype analysis to broaden our understanding of UC-MSC effects in COPD.
- The limitation of this study is that it was not conducted as a randomized control trial due to the complexity of the process and patient recruitment as well as the challenges of undertaking clinical trials in COPD patients due to the heterogeneity of disease mechanisms and phenotypic expression.

Introduction

Chronic obstructive pulmonary disease (COPD) is described – but not defined – as one of the major chronic lung diseases characterized by persistent and progressive airflow obstruction. It is caused by an elevated chronic pulmonary inflammatory response in the airways and bronchial structure to noxious particles or gases. The pathological hallmarks of the disease include obstructive bronchiolitis, emphysema, and mucus hypersecretion[1]. Despite many medical advancements and technological improvements, our understanding of the pathological mechanisms underlying the progressive and detrimental development of COPD remains incomplete, the definition of the disease is controversial, diagnostic tests are inaccurate and unstandardized, and the treatment is inadequate[2]. A recent report stated that the global prevalence of COPD increased by 44% within the last 20 years, and more than 3.2 million patients died each year from COPD worldwide (accounting for approximately 5% of all deaths globally per year) [3]. In Vietnam, according to the WHO report, 7.1% of males and 1.9% of females aged 40 and above are diagnosed with COPD. Consequently, approximately 25% of hospital beds in respiratory wards are required for COPD patients, resulting in a heavy burden to Vietnamese Medical Infrastructure and reducing patients' health and quality of life [4]. The current pharmacological medications for COPD include the use of inhaled bronchodilator drugs, such as long-acting β agonists (LABAs) and long-acting muscarinic antagonists (LAMAs), the use of inhaled corticosteroids (ICSs) or a combination of these medications. Although it is generally accepted that pharmacological interventions via inhalation would allow the accurate delivery of drugs and increase the clinical benefits, incorrect inhaler technique and a lack of adherence when feeling healthy caused worse dyspnea, impaired health condition, and increased the frequency of exacerbations and hospitalizations in Vietnamese COPD patients [5]. Therefore, identifying novel effective therapies for COPD patients is urgent and important.

Since their first discovery in 1968, mesenchymal stem/stromal cells (MSCs) have been intensively studied because of their therapeutic and regenerative features. The nomenclature of MSCs has been debated recently due to not only the biological features of the MSCs themselves but also the medical abuse of the term "stem cells" inferring direct medical benefit [6]. To standardize the characterization of MSCs and facilitate their therapeutic implications, the International Society for Cellular Therapy (ISCT) has proposed the minimum criteria to define human MSCs [7 8]. In our study, MSCs were defined as mesenchymal stem/stromal cells, which are a class of adult mesenchymal progenitor cells derived from either bone marrow, adipose, or umbilical cord tissue and met the minimum criteria of ISCT. Among various sources of MSCs, human umbilical cord-derived MSCs (hUC-MSCs) are potentially more advanced than their adult counterparts (bone marrow or adipose) for several reasons: (1) ease of collection as it is a noninvasive process, (2) waiving ethical barriers as UC is medical waste discarded at birth, (3) rapid proliferation rate, (4) maintenance of normal karyotype during prolonged culture *in vitro*, and (4) higher paracrine potency than adult tissue-derived MSCs [9]. The therapeutic potential of hUC-MSCs has been proven in clinical studies, especially animal pulmonary disease models, including acute respiratory distress syndrome (ARDS), bronchopulmonary dysplasia (BPD), and COPD. It has been reported that UC-MSCs are effective in reducing lung inflammation and fibrosis processes, preventing secondary infection, decreasing immune system damage, increasing bronchoalveolar fluid clearance, and enhancing the regeneration of alveolar epithelium layers [10-12]. The majority of intravenously

administered MSCs reportedly remain in the lung, especially pulmonary microvessels, which potentially contribute to their beneficial effects in pulmonary disease models [13]. Hence, the safety and therapeutic effects of UC-MSC administration for COPD require further investigation and clarification.

To date, five completed clinical trials have used bone marrow mononuclear cells (BMMCs), bone marrow-derived MSCs (BM-MSCs), and UC-MSCs in COPD [14-19]. Although these clinical trials provided an enormous amount of data supporting the safety of the therapy in the treatment of COPDs, the efficacy of the treatments remained controversial due to several limitations, including trial design, lack of standardization of cell numbers administered to patients, timing of MSC administration, and, most importantly, the lack of a control group in several studies. Moreover, the variations in patient selection based on the severity and stage of COPD could be attributed to the effectiveness of the cell therapy, resulting in caution in data interpretation. Last but not least, the quality of administered MSCs also plays a significant role in the effectiveness of the treatment, i.e., the status of the cells (fresh culture vs. frozen cells), cell sources (from young healthy donors or aging individuals), dosage frequency, etc. Therefore, identification of the potential sources of MSCs (such as UC-MSCs), larger sample size with matched controls, and standardized classification of COPD using international accepted criteria is required to further investigate the safety and efficacy of MSC therapy. Based on the large body of preclinical studies and previous promising findings, we designed a matched control phase I/II clinical trial to evaluate the safety and efficacy of the intravenous infusion of allogeneic hUC-MSCs in patients with COPD characterized based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2019 criteria[20].

Methods and analysis

Study objectives

The aim of this trial is to evaluate the safety and efficacy of allogeneic UC-MSC administration in patients with COPD. There are two specific objectives:

1. Evaluate the safety and potential therapeutic effectiveness of intravenously (IV) administered UC-MSCs in patients with COPD.
2. To prove the hypothesis that IV administration of UC-MSCs can improve lung function and reduce inflammatory responses in the lungs and fibrosis.
3. Explore the potential therapeutic mechanism of UC-MSCs in the treatment of COPD.

Study design and ethics

This matched case-control phase I/II clinical trial was approved by the Ethical Committee of Vinmec International Hospital (number: 166/2019/QĐ-VMEC) and Vietnam Ministry of Health (number: 2002/QĐ-BYT). This study was registered at ClinicalTrials.gov (number NCT04433104). To achieve the aims, a total of 40 patients with COPD will be recruited at the Internal Medicine Department at Vinmec Times City International Hospital, Hanoi, Vietnam, between June 2020 and December 2021. A flowchart of the study design is shown in Figure 1.

Sample size

As a previous study indicated that the mMRC score of COPD patients ranged from 18% to 60% reduction, we set this rate at 60% reduction to calculate the minimum sample size for the proposed study[18 21]. According to the dichotomous endpoint of two independent sample studies[22], we assumed α was 0.05 and type-II error β was 0.2; thus, the smallest sample size was 40 patients. The calculated sample size was 20 for each group.

Matching strategy:

The patients from the control group will be assigned to a patient from the MSC group once they meet all matched criteria based on age (±5 years), gender, and COPD severity classification (GOLD 2019). Patients from both groups will receive standard COPD medication management according to their COPD severity classification and based on the Vietnam Ministry of Health guideline for COPD treatment, as shown in Figure 2 and Table 3.

A total of 40 patients will be recruited and assigned to the UC-MSC administration group (20 patients) and the control group (20 patients). The trial contains two phases: (1) the first phase will include recruiting and evaluating the first 5 patients from each group to assess the safety of UC-MSC administration after 1 month of follow-up, and (2) the second phase will be initiated after the 1st phase safety report is approved by the Ethical Committee of Vinmec International Hospital and Vietnam Ministry of Health to start recruiting the remaining 15 patients from each group to evaluate both safety and efficacy of the treatment.

Participants

The principal investigators, research and clinician team members are responsible for the study design, patient screening, recruitment, conduct, and perform follow-up assessments in the trial. Participants will be allowed to enroll or withdraw at any time throughout the study. The participants will have all screening and testing costs related to the trials waived except for the costs of COPD medications or drugs. All participants' information will be protected by coding and restricted access using a computer-based system. Participants will be enrolled in the study once they meet all inclusion and exclusion criteria.

Inclusion and exclusion criteria

The diagnostic criteria and severity classification of COPD refers to the criteria established by the COPD 2019 guidelines [20]. Patients will be asked to confirm the COPD conditions and classification from national hospitals and send the results to the administration office prior to enrollment in the trial for prescreening. Patients will be enrolled in the study in compliance with the inclusion and exclusion criteria established by a screening protocol as presented below.

Inclusion Criteria

- Diagnosed with COPD with stage B, C, or D according to GOLD 2019.
- Age between 40-75 years old.
- Both genders.

Exclusion Criteria

- Smoker or less than 6 months of smoking cessation time.
- Asthma and other pulmonary-related diseases and injuries (including lung tuberculosis, restrictive lung disease, idiopathic pulmonary fibrosis, or lung cancer).
- Acute and/or active infection.
- Cancer.
- Patients with complex cardiovascular diseases (including valvular heart disease, cardiomyopathy, arrhythmia, congenital heart disease, hypertrophy syndrome).
- Liver and kidney failure.
- Pregnancy.
- Patients with life expectancy less than 6 months due to concomitant illness.
- Under immunosuppressive treatment within 8 weeks of the first screening visit.
- Patient diagnosed diabetes with $HbA_1C>7\%$.

Recruitment

Patients can only enroll in this study after passing the prescreening process, consultation resolution, and signing the informed consent form.

The recruitment campaign will target three main sources. First, potentially eligible hospitalized patients diagnosed with severe COPD will be approached and asked to participate in the study. Second, physicians will generate lists of patients from the electronic medical system of Vinmec Times City International Hospital with a diagnosis of COPD based on severity classification matching the GOLD 2019 criteria who were discharged within 2 years. Investigators or physicians will contact patients by telephone or mail them a research leaflet and recruitment letter. Third, leaflet and trial recruitment letters will be posted in the Vingroup cooperation internal email system, the official website, and the Facebook public platform of the Vinmec Healthcare system for those diagnosed with COPD GOLD 2019 (B, C, D) at other hospitals. If the patients are interested in this research, we will ask them to send the prescreen results to the administration office.

A multidisciplinary consultation will be held to evaluate the prescreening results from participants to confirm whether these potential participants meet the general diagnostic criteria of COPD, including inclusion and exclusion criteria. The consultation includes physicians and experts from respiratory, radiology, laboratory, and stem cell biology fields. If more than 80% of experts agree on the prescreening results, patients will be viewed as potential participants. The researchers will set an appointment to communicate with the potential candidates about the clinical trial details, including pros and cons of stem cell treatments and sign the written informed consent form prior to assigning patients to either stem cell administration or control groups.

The details of the clinical trial will be explained to patients by investigators or physicians as follows: (1) the study aims and scope, (2) background of COPD and UC-MSC, (3) number of participants, study duration, and classification into either MSC administration or control group, (4) study procedure (including screening, COPD medication management, follow-up tests), (5) potential discomfort and risks of MSC administration (including

prespecified adverse and severe adverse events), (6) expected outcomes of the treatment (primary safety evaluation and potential therapeutic improvement of both MSC administration and COPD medication management according to Vietnam Ministry of Health guideline), (7) protection policy of patients' information and privacy, and (8) voluntary participation (right and responsibility of patients). Patients will only sign written informed consent when all the above items are fully explained and the patients fully understand the protocol. The patients' baseline characteristics will be assessed by the clinicians within 30 days prior to UC-MSC administration for patients in the MSC administration group (Table 1).

Intervention

A single umbilical cord (UC) sample will obtained from healthy women with an uncomplicated, at term pregnancy who underwent serological testing, including tests for HIV, cytomegalovirus (CMV), Epstein-Barr virus (EBV), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis, and chlamydia, at 38 weeks of pregnancy. The eligible UC tissue will be collected at delivery and transferred to the Stem Cell Core Facility at the Vinmec Research Institute of Stem Cell and Gene Technology under ISO 14644-1 (certification number: CR61119-1). The UC tissue will be processed, and the UC-MSCs will be isolated and cultured under xeno-free and serum-free conditions as previously described. UC-MSCs will be expanded under these conditions to passage 5 (P5) and cryopreserved in the serum- and xeno-free defined reagent CryoStore® CS10 (Stem Cell Technology, Canada) in liquid nitrogen (gas phase) in an automated Brooks System (Brooks Life Science, USA) for long-term storage. The releasing criteria for UC-MSC products are shown in Table 2.

To prepare UC-MSCs for therapy, aliquots of P5 UC-MSCs will be thawed in a temperature control water bath or incubator on the infusion day. The hUC-MSCs will be washed and suspended in 0.9% normal saline. In addition to inspecting the quality of the UC-MSC product based on the releasing criteria, the staff of the Cell Therapy Department will confirm the viability and quality of the UC-MSC product before the infusion. The cell dose will be calculated based on patients' body weight and cell viability results to obtain the dose of $1x10^6$ viable cells/kg patient body weight prior to transport to the administration ward. Currently, there is no effective treatment for COPD patients. Thus, the intervention group will be given the standard COPD medication management as primary treatment and extra UC-MSC administration, while the control group will receive only the standard COPD treatment (Table 3).

Mode of cell administration (UC-MSC group)

Patients assigned to UC-MSC administration groups will receive two administrations at a dose of 1 million cells/kg patient bodyweight via the IV route with a 3-month intervening interval. On the day of infusion, thawed cells at P5 will be prepared to meet the target administration dose based on the number of viable cells in 10 mL of 0.9% NaCl (Braun, USA) as described above and delivered to the administration ward for infusion at a rate of 20 mL/hour.

Withdrawal

Participant discontinuation may occur upon participant death, severe adverse events (SEAs), other serious disease-limiting involvement, or a direct request from participant to withdraw from the study. Once the participant

withdraws from the study, the reasons for the withdrawal and all recorded results will be documented in detail. New participants will not be recruited to replace withdrawn participants.

Adverse events (AEs)

AEs are defined as adverse medical events that occur after the patient signs informed consent until completion of the follow-up period. AEs include abnormal laboratory results, symptoms, or diseases. All AEs will be documented on a written case report form (CRF) and transferred to a research electronic data capture (RedCap) system. Once AEs occur, the physician and clinician in charge will follow the necessary treatment according to the patient's condition and decide whether to suspend clinical research. In terms of severe adverse events (SAEs), the clinician team will follow the first priority to treat principle and be considered an emergency situation. The principal investigators will immediately inform the Ethical Committee and Medical Advisory board of Vinmec Times City International Hospital. Within 24 h, the SAE report should be submitted with full description, while a follow-up SAE report should be submitted to the Ethical Committee of Vinmec Times City International Hospital. Within the 7 days, the SAE report with comments from the Ethical Committee will be submitted to the National Ethical Committee of Vietnam Ministry of Health via post. All participants enrolled in the study will be subjected to an insurance policy that provides ancillary and posttrial medical care in case of injury or death as a result of their participation in the trial.

Outcome evaluation

Primary outcomes (safety)

All required evaluation and laboratory tests with the timeframes are listed in Table 1. To assess safety, the number of AEs or SAEs during stem cell administration (72 h) at 3 months, 6 months, and 12 months after discharge will be evaluated. Body temperature, blood pressure, respiratory rate, heart rate, and SpO_2 will be recorded before and during MSC administration up to 24 h. As mentioned above, the first phase of this study will involve recruiting five pairs of patients to evaluate the safety prior to initiating the second phase. The safety report of the first phase will cover one month postdischarge and will be submitted to the Ethical Committee of Vinmec Times City International Hospital and the National Ethical Committee of Vietnam Ministry of Health for approval of starting the second phase.

SAEs include death, any critical cardiac event (new ventricular tachycardia, ventricular fibrillation, or asystole, cardiac arrest, cardiac hypertrophy), acute pulmonary distress and embolism, stroke, anaphylactic shock, sepsis, and other conditions that extend the hospital stay. The prespecified AEs include fever, common allergic reactions (rash, edema, erythema, pallor), infection at the administration site, changes in vital signs, and abnormal laboratory test results (including hematological analysis and indicators of liver and kidney functions).

Secondary outcomes (Efficacy)

The efficacy endpoints are as follows: (1) number of admissions and readmissions, (2) general self-efficacy, (3) the number of admissions and unscheduled outpatient visits due to symptoms of COPD, (3) arterial blood gas analysis (including pH, PaO_2, $PaCO_2$, BE, HCO_3-), (4) respiratory functions (FEV_1, FEV_1/FVC, VC, TLC, RV, DLCO, DLNO/DLCO), (5) electrocardiogram, echocardiography, high-resolution chest computed tomography,

abdominal ultrasound, abnormality of thyroid and mammary gland, (6) inflammatory response (CRP, Pro-BNP, and Troponin-T) and (7) cytokine analysis from patients' plasma. In addition, the modified medical research council (mMRC) questionnaire and quality of life (Georges Respiratory Questionnaire – SGRQ) will be used to monitor respiratory function improvement. To reveal the therapeutic effects of UC-MSC administration, UC-MSC characterization will be conducted *in vitro*, including MSC marker analysis, metabolic evaluation, immunoregulatory assessment, and cytokine secretion analysis.

Follow-up procedure

Follow-up visits will be conducted at 3, 6, and 12 months after hUC-MSC administration. Patients will be asked to come to the hospital to undergo an assessment of their conditions according to the protocol procedure. The safety follow-up will include an extra 1-month follow-up point via telephone and outpatient contact, and patients will only be asked to make an appointment if AEs or SAEs occur.

Data collection

The data accumulated during the trial will be documented in the patients' medical records and the CRF. The quality control officers from Vinmec Times City International Hospital and Vinmec Scientific Research Board independently checked the accuracy and consistency of the CRF data with the original patients' medical records to ensure that the data were accurately entered into the CRF. Once the CRF is checked, within 7 days, all data will be recorded to RedCap software by assigned personnel and crosschecked by principle investigators. There are four data collection points, including baseline and 3 months, 6 months, and 12 months postadministration. The internal auditor of the Vinmec Research Institute of Stem Cell and Gene Technology will review each original research record to confirm the accuracy, consistency, timely records, and meet the standard requirements. Data analysis will be performed using RedCap and statistical analysis software following the statistical analysis strategy (https://redcap.vinmec.com/). The data of this clinical trial will be disseminated with permission from funding bodies and principle investigators through national and international conferences, peer-reviewed publications, and scientific reports. A complete data set will be available upon request after trial completion.

Statistical analysis strategy

Descriptive statistics will be used to illustrate the demographics of the COPD patients. Categorical variables are expressed as proportions, whereas quantitative variables are described as the mean values and their standard deviations or as the medians and their interquartile ranges. The number and type of adverse events/serious adverse events will be compared between the two treatment groups using the Chi square (or Fisher's exact) test. For the intervention and control groups, the indicators (m-MRC, CAT, SGRQ, respiratory functions, and arterial blood gas analysis) at baseline and at 3 months, 6 months, and 12 months will be compared with repeated measures ANOVA. P-values < 0.05 will be considered statistically significant. The analyses will be performed using Stata version 14 (StataCorp, College Station, TX, USA).

Ethics and dissemination

This trial, including the consent form and clinical protocol, was approved by the Ethical Committee of Vinmec International Hospital (number: 166/2019/QĐ-VMEC) and Vietnam Ministry of Health (number: 2002/QĐ-BYT). This study was registered at ClinicalTrials.gov (number NCT04433104). The trial conforms with the Declaration of Helsinki. All participants will provide oral and written informed consent prior to participating in the study.

Discussion

This study protocol presents the matched-control phase I/II clinical trial evaluating the safety and potential efficacy of allogeneic UC-MSC administration in patients with moderate-to-severe COPD (GOLD 2019). To date, there is no effective treatment available for COPD patients, and pharmacological interventions are hampered by the heterogeneity of disease mechanisms and phenotypic alternation. Therefore, establishing new treatment methods to reduce the devastating effects of COPD is imperative. The body of preclinical studies and human clinical trials suggests that MSC administration emerges as a potential therapeutic approach for COPD because MSCs have been found to be well tolerated and safe in many clinical trials and have proven their effectiveness in animal models. However, the effectiveness of MSC therapy showed differences among various clinical trials, and a small number of trials have revealed no significant changes in lung function and fibrosis postadministration compared with baseline levels[23]. Therefore, it is important to comprehensively analyze the factors that directly contribute to treatment efficacy.

Human clinical trials were conducted to evaluate the safety and efficacy of MSCs in the treatment of COPD, including five studies using bone marrow-derived cells [14-17] [19] and a pilot study using UC-MSCs[18]. The first study supported the safety profile of MSCs administered BMMCs to four COPD patients, although the overall clinical outcomes did not demonstrate the efficacy of the treatment. It is understandable that studies together with the two trials (NCT001110252 and NCT01306513) are phase 1 clinical trials that aimed to evaluate the safety and feasibility of cellular administration in the treatment of COPD. Notably, the NCT001110252 study followed up with patients for up to 3 years illustrated an overall reduction in the process of COPD pathological development [19]. In a pilot study using UC-MSCs, COPD patients were followed up for 6 months, and no AEs or SAEs were observed throughout the course of the study. Although clinical outcomes such as COPD exacerbations, mMRC score, and CAT were significantly reduced postadministration, pulmonary function parameters remained unchanged compared to baseline [18]. In our current study, we use UC-MSCs as an "off-the-shelf" product for administration, providing flexibility in patient management and standardized products for all treated patients, allowing more accuracy in evaluation. Moreover, by using a matched control design, our study aims to eliminate the variability in COPD conditions between the intervention and control groups to accurately evaluate the safety and efficacy of the treatment. In general, it was confirmed that MSC administration is well tolerated without serious adverse events or administration-associated adverse events and is not associated with significant alterations in spirometry, immune function, cardiovascular activity, or patient quality of life [24].

The major delivery routes of MSCs in the treatment of pulmonary disease include intraperitoneal (usually in animal models), intranasal or intratracheal, and IV administration. The intratracheal administration of MSCs was performed in children with bronchopulmonary dysplasia in several small uncontrolled studies. However, in terms of COPD, all trials utilized IV administration with the aim of investigating whether systemic administration of MSCs is

safe and effective in COPD patients. In fact, the IV administration route is considered a better option compared to intratracheal delivery for several reasons. Previous studies illustrated that IV administration of MSCs was safe and potentially provided therapeutic effects in several lung diseases, including COPD [15 23]. Moreover, a systemic analysis of preclinical studies suggested that IV administration of MSCs introduced better effects than those administered via the intratracheal route [13]. We hypothesized that the results of this clinical trial will provide data supporting that UC-MSC administration via the IV route is safe, feasible, and potentially effective in COPD patients.

The dose-escalating evaluation has been conducted in several clinical trials for various diseases, including pulmonary syndromes, using a wide range of UC-MSC doses from 0.5 – 10 million cells/kg via IV administration [15 23 25]. Notably, limited studies have reported the different effects of MSC doses in COPD patients. In fact, a relatively high dose (10 million cells/kg patient body weight) was tested in ARDS patients without any administration-associated AEs or SAEs recorded. However, it is important to note that delivery of a high dose of stem cells might increase the risk of pulmonary embolism and thrombosis regardless of administration route, which was demonstrated previously in animal models and clinical trials [26-28]. Therefore, in this trial, we used the most common dose of MSCs used in numerous studies, which is 1 million cells/kg patient body weight.

This clinical trial has several advantages. First, this is the first trial using an "off-the-shelf" product (UC-MSCs) for COPD patients. Second, this is the first trial to investigate the therapeutic effects of UC-MSCs as supplementary products in combination with standard medication treatments according to the GOLD 2019 recommendation. Third, if the potential efficacy can be detected throughout the course of our study, our results (including MSC biological analysis of stem cell characterization, immunoregulation, and metabolism) will strengthen our knowledge and understanding of UC-MSC effects in COPD and provide a fundamental background for treating patients with moderate-to-severe COPD. In the case of no therapeutic effect, our data will also provide important insight into the safety of the treatment and potential alternative approach for MSC therapy of COPD.

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Contributors:

TLN, DMH, and KTN were involved in the design of the study. DMH drafted the manuscripts with critical input from LNT, KTN, AHN, and BNN. LNT, AHN, and BNN contributed to the standard medical treatment checklist and drug for all patients. DMH, LNT and AHN are the grant holder and project leader, respectively. All authors reviewed, edited and approved the final version of the manuscript.

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Conflicts of Interests

None declared.

Patient and public involvement

The patients and public were not involved in the design, or conduct, or reporting or dissemination plans of our research.

Patient consent for publication

Not required

Figure Legend:

Figure 1: Schematic of the study. COPD patients will be screened to enroll in the study. Patients from the control group will be assigned to a patient from the UC-MSC group once they meet all matched criteria based on age (±5 years), gender, and COPD severity classification (GOLD 2019).

Figure 2: Standard COPD medication treatment for both groups according to GOLD 2019 and Vietnam Ministry of Health Guideline. Matched COPD patients will be treated using the same treatment based on their GOPD 2019 classification (Groups A, B, C, and D). Group A (not included in this study): a single bronchodilator will be used and based on the clinical assessments and persistence of the symptoms to continue/stop or replace by another bronchodilator. Group B: Single LAMA or LABA will be initially used. If the symptoms are not reduced, a combination of both LAMA and LABA will be applied. Group C: A single LAMA drug will be used for initial treatment. If exacerbations occur, LAMA and LABA combination will be applied as priority. The LAMA + ICS will be applied in specific cases based on clinical assessment, as the ICS has been reported to have severe side effects on lung inflammation. Group D: Should start the treatment with LAMA. If the patient has CAT>20, LABA and LAMA will be used as initial treatment. LABA + ICS will be used as the initial treatment only when the patient has asthma COPD overlap or the patient's eosinophil level > 300. If exacerbation occurs after the initial treatment, the combination of LAMA, LABA, and ICS should be applied. Additional roflumilast should be used if FEV1 < 50% and the patient has chronic bronchitis. Macrolide should be used if the patient is a former smoker. The red arrow indicates priority treatment.

Table 1: Study timeline and clinical procedures during the trial. * If the results of the screening phase for UC-MSC groups are within 30 days of UC-MSC administration, they will be automatically considered as the baseline level.

Study Procedure	Prescreening	Screening phase*	Baseline	3 months	6 months	12 months
UC-MSC administration[1]			☑	☑		
Medication treatment[2]			☑	☑	☑	☑
Informed consent		☑				
Inclusion and exclusion criteria		☑				
Demographic information		☑	☑			
Patients' medical reports		☑	☑	☑	☑	☑
Vital signs[3]/physical examination		☑	☑	☑	☑	☑
COPD assessment	☑	☑				
COPD GOLD 2019 classification	☑	☑		☑	☑	☑
Hematology analysis[4]	☑	☑	☑	☑	☑	☑
Infectious disease examination/test[5]	☑	☑	☑			
Blood oxygen saturation/arterial blood gas analysis[6]	☑	☑	☑	☑	☑	☑
Chest CT scan		☑	☑		☑	☑
Chest X-ray		☑	☑		☑	☑
Pulmonary function analysis		☑	☑	☑	☑	☑
Adverse event evaluation			☑	☑	☑	☑
Mortality/complications monitoring			☑	☑	☑	☑

[1]: Applies only for the UC-MSC group at baseline and 3 months.

[2]: Treatment medication applies for all testing groups based on patients' COPD classification according to GOLD 2019 guidelines.

[3]: Vital signs include body temperature, blood pressure, heart rate, respiratory rate, oxygen saturation, and patient body weight.

[4]: Hematological analysis included white blood cell count, platelet count, red blood cell count, hemoglobin, percentage of lymphocytes, neutrophils, monocytes, eosinophils, basophils, C-reactive protein, Pro-BNP, and Troponin-T.

[5]: Infectious diseases include hepatitis, syphilis, HIV, HBV, and tuberculosis.

[6]: Blood gas analysis includes pH, PaO_2, $PaCO_2$, BE, HCO_3-.

Table 2: Release criteria and stem cell quality control. To assess the quality of UC-MSCs for administration, a set of release criteria was defined, which included the following: the positive markers (CD73, CD90, and CD105) must be higher than 95%, the negative markers (CD11b, CD19, CD34, CD45, and HLA-DR) must be less than 2%; the cell viability must be higher than 80% with a normal karyotype; and the cell product must be free from microorganism infections and mycoplasma. Immunoregulatory assays will be performed to assess but not consider released criteria.

Criteria	Testing Method	Released criteria
Positive markers (%) (median, range)		
CD73	Flow cytometry using the Human MSC Analysis Kit (Becton Dickinson,USA)	> 95%
CD90		> 95%
CD105		> 95%
Negative markers (%)		< 2%
Cell viability (%) (mean ± SD)	Trypan Blue staining	> 80%
Microorganism tests	BacT/Alert® 3D microbial detection System (Biomerieux, USA)	Negative
Mycoplasma	MycoAlertTM Plus Mycoplasma Detection Kit (Lonza, Switzerland)	Negative
Endotoxin	Endosafe-PTS (Charles River Laboratories)	≤ 5 EU/kg
Immunoregulatory assay	Flow Cytometry	Not Applicable

Table 3: Standard medication treatment for both groups based on GOLD 2019 guidelines and Vietnam Ministry of Health recommendations.

Items	COPD GOLD 2019 Group B	COPD GOLD 2019 Group C	COPD GOLD 2019 Group D
Initial treatment	A long acting bronchodilator (LABA or LAMA)	LAMA	LAMA
Difficulty in breathing (moderate)	LAMA + LABA	LAMA + LABA Or LAMA + ICS	LAMA + LABA ISC/LABA use when: ▪ Asthma COPD overlap. ▪ Eosinophils>300/ul.
Difficulty in breathing (Severe)	LAMA + LABA	LAMA + LABA Or LAMA + ICS	LAMA + LABA + ICS
Name of Drugs use in Standard COPD Medication Treatment for both groups			
SABA	Salbutamol, Terbutaline, Fenoterol		
LABA	Indacaterol, Bambuterol		
SAMA	Ipratropium		
LAMA	Tiotropium		
SABA + SAMA	Ipratropium and salbutamol Ipratropium and fenoterol		
LABA + LAMA	Indacaterol and Glycopyronium Olodaterol and Tiotropium Vilanterol and Umeclidinium		
ICS + LABA	Budesonid and Formoterol Fluticason and Vilanterol Fluticason and Salmeterol		
Antibiotics	Erythromycin Rofumilast[1]		
Long/short-acting Xanthine	Theophyllin/Theostat		

[1]: Roflumilast was used only when patients' FEV1<50% and had at least 1 admission within 1 year.

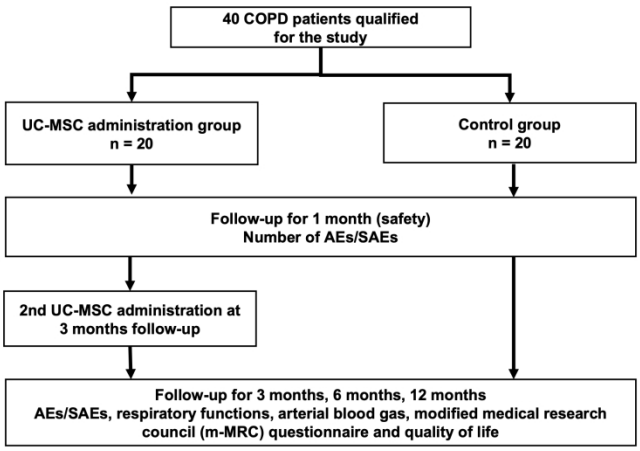

Figure 1

Figure 1: Schematic of the study. COPD patients will be screened to enroll in the study. Patients from the control group will be assigned to a patient from the UC-MSC group once they meet all matched criteria based on age (±5 years), gender, and COPD severity classification (GOLD 2019).

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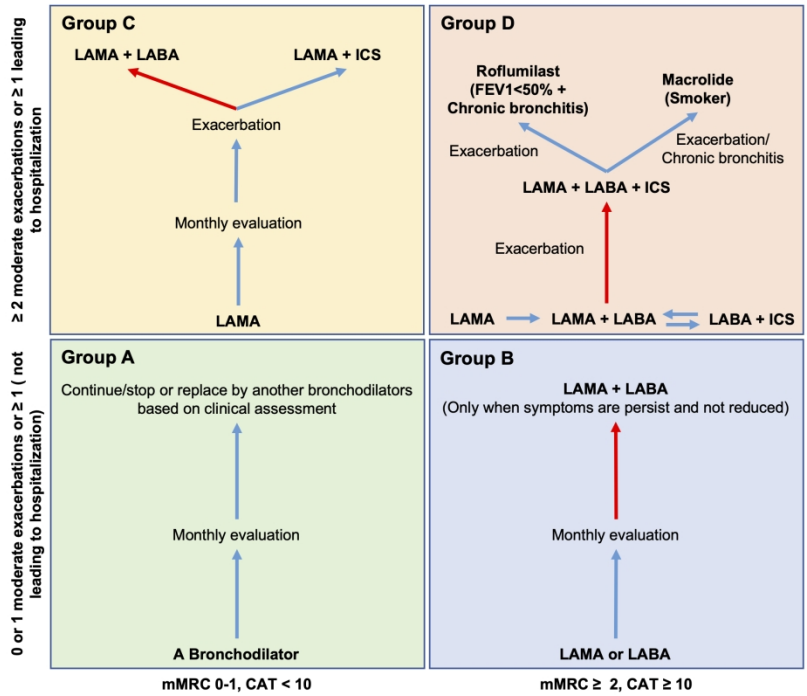

Figure 2:

Figure 2: Standard COPD medication treatment for both groups according to GOLD 2019 and Vietnam Ministry of Health Guideline. Matched COPD patients will be treated using the same treatment based on their GOPD 2019 classification (Groups A, B, C, and D). Group A (not included in this study): a single bronchodilator will be used and based on the clinical assessments and persistence of the symptoms to continue/stop or replace by another bronchodilator. Group B: Single LAMA or LABA will be initially used. If the symptoms are not reduced, a combination of both LAMA and LABA will be applied. Group C: A single LAMA drug will be used for initial treatment. If exacerbations occur, LAMA and LABA combination will be applied as priority. The LAMA + ICS will be applied in specific cases based on clinical assessment, as the ICS has been reported to have severe side effects on lung inflammation. Group D: Should start the treatment with LAMA. If the patient has CAT>20, LABA and LAMA will be used as initial treatment. LABA + ICS will be used as the initial treatment only when the patient has asthma COPD overlap or the patient's eosinophil level > 300. If exacerbation occurs after the initial treatment, the combination of LAMA, LABA, and ICS should be applied. Additional roflumilast should be used if FEV1 < 50% and the patient has chronic bronchitis. Macrolide should be used if the patient is a former smoker. The red arrow indicates priority

treatment.

190x275mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	"n/a"
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	14
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	14

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	14
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4-5
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5, 6
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5, 6

Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6,7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	8, 9
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	8, 9
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8, 9
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9, 10
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	5,6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7,8
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be	"n/a"

		provided in a separate document that is unavailable to those who enrol participants or assign interventions	
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10

Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	10
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	10
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	9, 10
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	10, 11
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5, 6

Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	5,6
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	10
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	"n/a"
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	10
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	14
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	"n/a"
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	"n/a"
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Table 2

BMJ Open

Allogeneic human umbilical cord-derived mesenchymal stem/stromal cells for chronic obstructive pulmonary disease (COPD): study protocol for a matched case-control, phase I/II trial

Journal:	*BMJ Open*
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Complete List of Authors:	Hoang, Duc M.; Vinmec Health Care System, Vinmec Research Institute of Stem Cell and Gene Technology, Cellular Manufacturing Department Nguyen, Kien T.; Vinmec Health Care System, Vinmec Research Institute of Stem Cell and Gene Technology, Scientific Research Department Nguyen, Anh H.; Vinmec Times City International Hospital, Department of Internal Medicine Nguyen, Bach N.; Vinmec Times City International Hospital, Department of Internal Medicine Nguyen, Liem; Vinmec Health Care System, Vinmec Research Institute of Stem Cell and Gene Technology
Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Research methods
Keywords:	Transplant medicine < INTERNAL MEDICINE, RESPIRATORY MEDICINE (see Thoracic Medicine), Chronic airways disease < THORACIC MEDICINE

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Allogeneic human umbilical cord-derived mesenchymal stem/stromal cells for chronic obstructive pulmonary disease (COPD): study protocol for a matched case-control, phase I/II trial.

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Keywords: Umbilical cord-derived Mesenchymal Stem/stromal Cells, Chronic Obstructive Pulmonary Disease (COPD), allogeneic MSC administration, clinical trial.

Word count: 4282 words

Abstract

Introduction

The global prevalence of chronic obstructive pulmonary disease (COPD) is increasing, and it has become a major public health burden worldwide, including in Vietnam. A large body of preclinical and clinical studies supports the safety of mesenchymal stem/stromal cells (MSCs) in the treatment of lung injury, including COPD. The aim of this trial is to investigate the safety and potential therapeutic efficacy of allogeneic administration of umbilical cord-derived MSCs (UC-MSCs) as a supplementary intervention in combination with standard COPD medication treatments in patients with moderate-to-severe COPD based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2019 and Vietnam Ministry of Health's guidelines.

Methods and analysis

This matched case-control phase I/II trial is conducted at Vinmec Times City International Hospital, Hanoi, Vietnam between June 2020 and December 2021. In this study, 40 patients will be enrolled and assigned into two age-, gender- and COPD condition-matched groups, including a UC-MSC group and a control group. Both groups will receive standard COPD medication treatment based on the GOLD 2019 guidelines and the Vietnam Ministry of Health protocol. The UC-MSC group will receive two doses of thawed UC-MSC product with an intervention interval of 3 months. The primary outcome measures will include the incidence of prespecified administration-associated adverse events (AEs) and serious adverse events (SAEs). The efficacy will be evaluated based on the absolute changes in the number of admissions, arterial blood gas analysis, lung function and lung fibrosis via CT scan and chest X-ray. The clinical evaluation will be conducted at baseline and 3, 6, and 12 months post intervention.

Ethics and dissemination

Ethical approval was secured from the Ethical Committee of Vinmec International Hospital (number:166/2019/QĐ-VMEC) and Vietnam Ministry of Health (number:2002/QĐ-BYT). The results will be reported to trial collaborators, publication in peer-reviewed academic journals.

Trial registration number:NCT04433104.

Strengths and Limitations

- This project is the first matched case-control phase I/II study to evaluate the safety and efficacy of allogeneic administration of UC-MSCs as supplementary treatment in combination with standard medication treatments for patients with moderate-to-severe COPD.
- To address the challenge of evaluating the effectiveness of MSC treatment in COPD by using quantitative and qualitative research methods.
- To interlink the treatment effectiveness with stem cell phenotype analysis to broaden our understanding of UC-MSC effects in COPD.
- The limitation of this study is that it was not conducted as a randomized control trial due to the complexity of the process and patient recruitment as well as the challenges of undertaking clinical trials in COPD patients due to the heterogeneity of disease mechanisms and phenotypic expression.

Introduction

Chronic obstructive pulmonary disease (COPD) is described – but not defined – as one of the major chronic lung diseases characterized by persistent and progressive airflow obstruction. It is caused by an elevated chronic pulmonary inflammatory response in the airways and bronchial structure to noxious particles or gases. The pathological hallmarks of the disease include obstructive bronchiolitis, emphysema, and mucus hypersecretion[1]. Despite many medical advancements and technological improvements, our understanding of the pathological mechanisms underlying the progressive and detrimental development of COPD remains incomplete, the definition of the disease is controversial, diagnostic tests are inaccurate and unstandardized, and the treatment is inadequate[2]. A recent report stated that the global prevalence of COPD increased by 44% within the last 20 years, and more than 3.2 million patients died each year from COPD worldwide (accounting for approximately 5% of all deaths globally per year) [3]. In Vietnam, according to the WHO report, 7.1% of males and 1.9% of females aged 40 and above are diagnosed with COPD. Consequently, approximately 25% of hospital beds in respiratory wards are required for COPD patients, resulting in a heavy burden to Vietnamese Medical Infrastructure and reducing patients' health and quality of life [4]. The current pharmacological medications for COPD include the use of inhaled bronchodilator drugs, such as long-acting β agonists (LABAs) and long-acting muscarinic antagonists (LAMAs), the use of inhaled corticosteroids (ICSs) or a combination of these medications. Although it is generally accepted that pharmacological interventions via inhalation would allow the accurate delivery of drugs and increase the clinical benefits, incorrect inhaler technique and a lack of adherence when feeling healthy caused worse dyspnea, impaired health condition, and increased the frequency of exacerbations and hospitalizations in Vietnamese COPD patients [5]. Therefore, identifying novel effective therapies for COPD patients is urgent and important.

Since their first discovery in 1968, mesenchymal stem/stromal cells (MSCs) have been intensively studied because of their therapeutic and regenerative features. The nomenclature of MSCs has been debated recently due to not only the biological features of the MSCs themselves but also the medical abuse of the term "stem cells" inferring direct medical benefit [6]. To standardize the characterization of MSCs and facilitate their therapeutic implications, the International Society for Cellular Therapy (ISCT) has proposed the minimum criteria to define human MSCs [7 8]. In our study, MSCs were defined as mesenchymal stem/stromal cells, which are a class of adult mesenchymal progenitor cells derived from either bone marrow, adipose, or umbilical cord tissue and met the minimum criteria of ISCT. Among various sources of MSCs, human umbilical cord-derived MSCs (hUC-MSCs) are potentially more advanced than their adult counterparts (bone marrow or adipose) for several reasons: (1) ease of collection as it is a noninvasive process, (2) waiving ethical barriers as UC is medical waste discarded at birth, (3) rapid proliferation rate, (4) maintenance of normal karyotype during prolonged culture *in vitro*, and (4) higher paracrine potency than adult tissue-derived MSCs [9]. The therapeutic potential of hUC-MSCs has been proven in clinical studies, especially animal pulmonary disease models, including acute respiratory distress syndrome (ARDS), bronchopulmonary dysplasia (BPD), and COPD. It has been reported that UC-MSCs are effective in reducing lung inflammation and fibrosis processes, preventing secondary infection, decreasing immune system damage, increasing bronchoalveolar fluid clearance, and enhancing the regeneration of alveolar epithelium layers [10-12]. The majority of intravenously

administered MSCs reportedly remain in the lung, especially pulmonary microvessels, which potentially contribute to their beneficial effects in pulmonary disease models [13]. Hence, the safety and therapeutic effects of UC-MSC administration for COPD require further investigation and clarification.

To date, five completed clinical trials have used bone marrow mononuclear cells (BMMCs), bone marrow-derived MSCs (BM-MSCs), and UC-MSCs in COPD [14-19]. Although these clinical trials provided an enormous amount of data supporting the safety of the therapy in the treatment of COPDs, the efficacy of the treatments remained controversial due to several limitations, including trial design, lack of standardization of cell numbers administered to patients, timing of MSC administration, and, most importantly, the lack of a control group in several studies. Moreover, the variations in patient selection based on the severity and stage of COPD could be attributed to the effectiveness of the cell therapy, resulting in caution in data interpretation. Last but not least, the quality of administered MSCs also plays a significant role in the effectiveness of the treatment, i.e., the status of the cells (fresh culture vs. frozen cells), cell sources (from young healthy donors or aging individuals), dosage frequency, etc. Therefore, identification of the potential sources of MSCs (such as UC-MSCs), larger sample size with matched controls, and standardized classification of COPD using international accepted criteria is required to further investigate the safety and efficacy of MSC therapy. Based on preclinical studies and previous promising findings, we designed a matched control phase I/II clinical trial to evaluate the safety and potential efficacy of the intravenous infusion of allogeneic hUC-MSCs in patients with COPD characterized based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2019 criteria[20].

Methods and analysis

Study objectives

The aim of this trial is to evaluate the safety and potential efficacy of allogeneic UC-MSC administration in patients with COPD. There are three specific objectives:

1. Evaluate the safety and potential therapeutic effectiveness of intravenously (IV) administered UC-MSCs in patients with COPD.
2. To prove the hypothesis that IV administration of UC-MSCs can improve lung function and reduce inflammatory responses in the lungs and fibrosis.
3. Explore the potential therapeutic mechanism of UC-MSCs in the treatment of COPD.

Study design and ethics

This matched case-control phase I/II clinical trial was approved by the Ethical Committee of Vinmec International Hospital (number: 166/2019/QĐ-VMEC) and Vietnam Ministry of Health (number: 2002/QĐ-BYT). This study was registered at ClinicalTrials.gov (number NCT04433104). To achieve the aims, a total of 40 patients with COPD will be recruited at the Internal Medicine Department at Vinmec Times City International Hospital, Hanoi, Vietnam, between June 2020 and December 2021. A flowchart of the study design is shown in Figure 1.

Sample size

As a previous study indicated that the FEV_1 (%) of COPD patients was reduced to 35.4±7.1% (6% reduction) after 6 months post-administration, we set this indicator at 18% reduction after 12 months post-administration to calculate the minimum sample size for the proposed study[18 21]. According to the continuous endpoint of two independent sample studies[22], we assumed α was 0.05 and type-II error β was 0.2; thus, the smallest sample size was 40 patients. The calculated sample size was 20 for each group.

Matching strategy:

The patients from the control group will be assigned to a patient from the MSC group once they meet all matched criteria based on age (±5 years), gender, and COPD severity classification (GOLD 2019). Patients from both groups will receive standard COPD medication management according to their COPD severity classification and based on the Vietnam Ministry of Health guideline for COPD treatment, as shown in Figure 2 and Table 1.

A total of 40 patients will be recruited and assigned to the UC-MSC administration group (20 patients) and the control group (20 patients). The trial contains two phases: (1) the first phase will include recruiting and evaluating the first 5 patients from each group to assess the safety of UC-MSC administration after 1 month of follow-up, and (2) the second phase will be initiated after the 1st phase safety report is approved by the Ethical Committee of Vinmec International Hospital and Vietnam Ministry of Health to start recruiting the remaining 15 patients from each group to evaluate both safety and efficacy of the treatment.

Participants

The principal investigators, research and clinician team members are responsible for the study design, patient screening, recruitment, conduct, and perform follow-up assessments in the trial. Participants will be allowed to enroll or withdraw at any time throughout the study. The participants will have all screening and testing costs related to the trials waived except for the costs of COPD medications or drugs. All participants' information will be protected by coding and restricted access using a computer-based system. Participants will be enrolled in the study once they meet all inclusion and exclusion criteria.

Inclusion and exclusion criteria

The diagnostic criteria and severity classification of COPD refers to the criteria established by the COPD 2019 guidelines [20]. Patients will be asked to confirm the COPD conditions and classification from national hospitals and send the results to the administration office prior to enrollment in the trial for prescreening. Patients will be enrolled in the study in compliance with the inclusion and exclusion criteria established by a screening protocol as presented below.

Inclusion Criteria

- Diagnosed with COPD with stage B, C, or D according to GOLD 2019.
- Age between 40-75 years old.
- Both genders.

Exclusion Criteria

- Smoker or less than 6 months of smoking cessation time.
- Asthma and other pulmonary-related diseases and injuries (including lung tuberculosis, restrictive lung disease, idiopathic pulmonary fibrosis, or lung cancer).
- Acute and/or active infection.
- Cancer.
- Patients with complex cardiovascular diseases (including valvular heart disease, cardiomyopathy, arrhythmia, congenital heart disease, hypertrophy syndrome).
- Liver and kidney failure.
- Pregnancy.
- Patients with life expectancy less than 6 months due to concomitant illness.
- Under immunosuppressive treatment within 8 weeks of the first screening visit.
- Patient diagnosed diabetes with $HbA_1C>7\%$.

Recruitment

Patients can only enroll in this study after passing the prescreening process, consultation resolution, and signing the informed consent form.

The recruitment campaign will target three main sources. First, potentially eligible hospitalized patients diagnosed with severe COPD will be approached and asked to participate in the study. Second, physicians will generate lists of patients from the electronic medical system of Vinmec Times City International Hospital with a diagnosis of COPD based on severity classification matching the GOLD 2019 criteria who were discharged within 2 years. Investigators or physicians will contact patients by telephone or mail them a research leaflet and recruitment letter. Third, leaflet and trial recruitment letters will be posted in the Vingroup cooperation internal email system, the official website, and the Facebook public platform of the Vinmec Healthcare system for those diagnosed with COPD GOLD 2019 (B, C, D) at other hospitals. If the patients are interested in this research, we will ask them to send the prescreen results to the administration office.

A multidisciplinary consultation will be held to evaluate the prescreening results from participants to confirm whether these potential participants meet the general diagnostic criteria of COPD, including inclusion and exclusion criteria. The consultation includes physicians and experts from respiratory, radiology, laboratory, and stem cell biology fields. If more than 80% of experts agree on the prescreening results, patients will be viewed as potential participants. The researchers will set an appointment to communicate with the potential candidates about the clinical trial details, including pros and cons of stem cell treatments and sign the written informed consent form prior to assigning patients to either stem cell administration or control groups.

The details of the clinical trial will be explained to patients by investigators or physicians as follows: (1) the study aims and scope, (2) background of COPD and UC-MSC, (3) number of participants, study duration, and classification into either MSC administration or control group, (4) study procedure (including screening, COPD

medication management, follow-up tests), (5) potential discomfort and risks of MSC administration (including prespecified adverse and severe adverse events), (6) expected outcomes of the treatment (primary safety evaluation and potential therapeutic improvement of both MSC administration and COPD medication management according to Vietnam Ministry of Health guideline), (7) protection policy of patients' information and privacy, and (8) voluntary participation (right and responsibility of patients). Patients will only sign written informed consent when all the above items are fully explained and the patients fully understand the protocol. The patients' baseline characteristics will be assessed by the clinicians within 30 days prior to UC-MSC administration for patients in the MSC administration group (Table 2).

Intervention

30 Umbilical cord (UC) samples were obtained from healthy women with an uncomplicated, at term pregnancy who underwent serological testing, including tests for HIV, cytomegalovirus (CMV), Epstein-Barr virus (EBV), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis, and chlamydia, at 38 weeks of pregnancy, as shown in previous study[23]. The UC tissues were collected at delivery and transferred to the Stem Cell Core Facility at the Vinmec Research Institute of Stem Cell and Gene Technology under ISO 14644-1 (certification number: CR61119-1). To generate a UC-MSC line for the current study, a single eligible UC tissue will be processed, isolated and cultured under xeno-free and serum-free conditions as previously described[23]. UC-MSCs will be expanded under these conditions to passage 5 (P5) and cryopreserved in the serum- and xeno-free defined reagent CryoStore® CS10 (Stem Cell Technology, Canada) in liquid nitrogen (gas phase) in an automated Brooks System (Brooks Life Science, USA) for long-term storage. The releasing criteria for UC-MSC products are shown in Table 3.

To prepare UC-MSCs for therapy, aliquots of P5 UC-MSCs will be thawed in a temperature control water bath or incubator on the infusion day. The hUC-MSCs will be washed and suspended in 0.9% normal saline. In addition to inspecting the quality of the UC-MSC product based on the releasing criteria, the staff of the Cell Therapy Department will confirm the viability and quality of the UC-MSC product before the infusion. The cell dose will be calculated based on patients' body weight and cell viability results to obtain the dose of $1x10^6$ viable cells/kg patient body weight prior to transport to the administration ward. Currently, there is no effective treatment for COPD patients. Thus, the intervention group will be given the standard COPD medication management as primary treatment and extra UC-MSC administration, while the control group will receive only the standard COPD treatment (Table 1).

Mode of cell administration (UC-MSC group)

Patients assigned to UC-MSC administration groups will receive two administrations at a dose of 1 million cells/kg patient bodyweight via the IV route with a 3-month intervening interval. On the day of infusion, thawed cells at P5 will be prepared to meet the target administration dose based on the number of viable cells in 10 mL of 0.9% NaCl (Braun, USA) as described above and delivered to the administration ward for infusion at a rate of 20 mL/hour.

Withdrawal

Participant discontinuation may occur upon participant death, severe adverse events (SEAs), other serious disease-limiting involvement, or a direct request from participant to withdraw from the study. Once the participant withdraws from the study, the reasons for the withdrawal and all recorded results will be documented in detail. New participants will not be recruited to replace withdrawn participants.

Adverse events (AEs)

AEs are defined as adverse medical events that occur after the patient signs informed consent until completion of the follow-up period. AEs include abnormal laboratory results, symptoms, or diseases. All AEs will be documented on a written case report form (CRF) and transferred to a research electronic data capture (RedCap) system. Once AEs occur, the physician and clinician in charge will follow the necessary treatment according to the patient's condition and decide whether to suspend clinical research. In terms of severe adverse events (SAEs), the clinician team will follow the first priority to treat principle and be considered an emergency situation. The principal investigators will immediately inform the Ethical Committee and Medical Advisory board of Vinmec Times City International Hospital. Within 24 h, the SAE report should be submitted with full description, while a follow-up SAE report should be submitted to the Ethical Committee of Vinmec Times City International Hospital. Within the 7 days, the SAE report with comments from the Ethical Committee will be submitted to the National Ethical Committee of Vietnam Ministry of Health via post. All participants enrolled in the study will be subjected to an insurance policy that provides ancillary and posttrial medical care in case of injury or death as a result of their participation in the trial.

Outcome evaluation

Primary outcomes (safety)

All required evaluation and laboratory tests with the timeframes are listed in Table 1. To assess safety, the number of AEs or SAEs during stem cell administration (72 h) at 3 months, 6 months, and 12 months after discharge will be evaluated. Body temperature, blood pressure, respiratory rate, heart rate, and SpO_2 will be recorded before and during MSC administration up to 24 h. As mentioned above, the first phase of this study will involve recruiting five pairs of patients to evaluate the safety prior to initiating the second phase. The safety report of the first phase will cover one month postdischarge and will be submitted to the Ethical Committee of Vinmec Times City International Hospital and the National Ethical Committee of Vietnam Ministry of Health for approval of starting the second phase.

SAEs include death, any critical cardiac event (new ventricular tachycardia, ventricular fibrillation, or asystole, cardiac arrest, cardiac hypertrophy), acute pulmonary distress and embolism, stroke, anaphylactic shock, sepsis, and other conditions that extend the hospital stay. The prespecified AEs include fever, common allergic reactions (rash, edema, erythema, pallor), infection at the administration site, changes in vital signs, and abnormal laboratory test results (including hematological analysis and indicators of liver and kidney functions).

Secondary outcomes (Efficacy)

The efficacy endpoints are as follows: (1) number of admissions and readmissions, (2) general self-efficacy, (3) the number of admissions and unscheduled outpatient visits due to symptoms of COPD, (3) arterial blood gas

analysis (including pH, PaO_2, $PaCO_2$, BE, HCO_3-), (4) respiratory functions (FEV_1, FEV_1/FVC, VC, TLC, RV, DLCO, DLNO/DLCO), (5) electrocardiogram, echocardiography, high-resolution chest computed tomography, abdominal ultrasound, abnormality of thyroid and mammary gland, (6) inflammatory response (CRP, Pro-BNP, and Troponin-T) and (7) cytokine analysis from patients' plasma. In addition, the modified medical research council (mMRC) questionnaire and quality of life (Georges Respiratory Questionnaire – SGRQ) will be used to monitor respiratory function improvement. To reveal the therapeutic effects of UC-MSC administration, UC-MSC characterization will be conducted *in vitro*, including MSC marker analysis, metabolic evaluation, immunoregulatory assessment, and cytokine secretion analysis (Table 4).

Follow-up procedure

Follow-up visits will be conducted at 3, 6, and 12 months after hUC-MSC administration. Patients will be asked to come to the hospital to undergo an assessment of their conditions according to the protocol procedure. The safety follow-up will include an extra 1-month follow-up point via telephone and outpatient contact, and patients will only be asked to make an appointment if AEs or SAEs occur.

Data collection

The data accumulated during the trial will be documented in the patients' medical records and the CRF. The quality control officers from Vinmec Times City International Hospital and Vinmec Scientific Research Board independently checked the accuracy and consistency of the CRF data with the original patients' medical records to ensure that the data were accurately entered into the CRF. Once the CRF is checked, within 7 days, all data will be recorded to RedCap software by assigned personnel and crosschecked by principle investigators. There are four data collection points, including baseline and 3 months, 6 months, and 12 months postadministration. The internal auditor of the Vinmec Research Institute of Stem Cell and Gene Technology will review each original research record to confirm the accuracy, consistency, timely records, and meet the standard requirements. Data analysis will be performed using RedCap and statistical analysis software following the statistical analysis strategy (https://redcap.vinmec.com/). The data of this clinical trial will be disseminated with permission from funding bodies and principle investigators through national and international conferences, peer-reviewed publications, and scientific reports. A complete data set will be available upon request after trial completion.

Statistical analysis strategy

Descriptive statistics will be used to illustrate the demographics of the COPD patients. Categorical variables are expressed as proportions, whereas quantitative variables are described as the mean values and their standard deviations or as the medians and their interquartile ranges. The number and type of adverse events/serious adverse events will be compared between the two treatment groups using the Chi square (or Fisher's exact) test. For the intervention and control groups, the indicators (m-MRC, CAT, SGRQ, respiratory functions, and arterial blood gas analysis) at baseline and at 3 months, 6 months, and 12 months will be compared with repeated measures ANOVA. P-values < 0.05 will be considered statistically significant. The analyses will be performed using Stata version 14 (StataCorp, College Station, TX, USA).

Patient and public involvement

The patients and public were not involved in the design, or conduct, or reporting or dissemination plans of our research.

Ethics and Dissemination

This trial, including the consent form and clinical protocol, was approved by the Ethical Committee of Vinmec International Hospital (number: 166/2019/QĐ-VMEC) and Vietnam Ministry of Health (number: 2002/QĐ-BYT). This study was registered at ClinicalTrials.gov (number NCT04433104). The trial conforms with the Declaration of Helsinki. All participants will provide oral and written informed consent prior to participating in the study. This study will be reported in accordance with the STROBE guidelines for matched case-control trial[24]. We will disseminate the research results through high-quality peer-reviewed open access (via PubMed) journals and presentations at national and international conferences. Finally, an ongoing update of the trial will also be provided and shared annually with our partners in the health system and community agencies according to National Regulation.

Discussion

This study protocol presents the matched-control phase I/II clinical trial evaluating the safety and potential efficacy of allogeneic UC-MSC administration in patients with moderate-to-severe COPD (GOLD 2019). To date, there is no effective treatment available for COPD patients, and pharmacological interventions are hampered by the heterogeneity of disease mechanisms and phenotypic alternation. Therefore, establishing new treatment methods to reduce the devastating effects of COPD is imperative. The body of preclinical studies and human clinical trials suggests that MSC administration emerges as a potential therapeutic approach for COPD because MSCs have been found to be well tolerated and safe in many clinical trials and have proven their effectiveness in animal models. Several clinical trials have been conducted in COPD. Most of these studies were phase 1 safety trials, which uniformly reported no obvious adverse events and serious adverse events as well as no evidence of infusional toxicities during the follow-up period [25]. However, the effectiveness of MSC therapy showed differences among various clinical trials, and a small number of trials have revealed no significant changes in lung function and fibrosis postadministration compared with baseline levels[26]. Therefore, it is important to comprehensively analyze the factors that directly contribute to treatment safety and efficacy.

Human clinical trials were conducted to evaluate the safety and efficacy of MSCs in the treatment of COPD, including five studies using bone marrow-derived cells [14-17 19] and a pilot study using UC-MSCs[18]. The first study supported the safety profile of MSCs administered BMMCs to four COPD patients, although the overall clinical outcomes did not demonstrate the efficacy of the treatment. It is understandable that studies together with the two trials (NCT001110252 and NCT01306513) are phase 1 clinical trials that aimed to evaluate the safety and feasibility of cellular administration in the treatment of COPD. Notably, the NCT001110252 study followed up with patients for up to 3 years illustrated an overall reduction in the process of COPD pathological development [19]. In a pilot study using UC-MSCs, COPD patients were followed up for 6 months, and no AEs or SAEs were observed throughout the course of the study. Although clinical outcomes such as COPD exacerbations, mMRC score, and CAT were

significantly reduced postadministration, pulmonary function parameters remained unchanged compared to baseline [18]. In our current study, we use UC-MSCs as an "off-the-shelf" product for administration, providing flexibility in patient management and standardized products for all treated patients, allowing more accuracy in evaluation. Moreover, by using a matched control design, our study aims to eliminate the variability in COPD conditions between the intervention and control groups to accurately evaluate the safety and efficacy of the treatment. In general, it was confirmed that MSC administration is well tolerated without serious adverse events or administration-associated adverse events and is not associated with significant alterations in spirometry, immune function, cardiovascular activity, or patient quality of life [27].

The major delivery routes of MSCs in the treatment of pulmonary disease include intraperitoneal (usually in animal models), intranasal or intratracheal, and IV administration. The intratracheal administration of MSCs was performed in children with bronchopulmonary dysplasia in several small uncontrolled studies. However, in terms of COPD, all trials utilized IV administration with the aim of investigating whether systemic administration of MSCs is safe and effective in COPD patients. In fact, the IV administration route is considered a better option compared to intratracheal delivery for several reasons. Previous studies illustrated that IV administration of MSCs was safe and potentially provided therapeutic effects in several lung diseases, including COPD [15 26]. Moreover, a systemic analysis of preclinical studies suggested that IV administration of MSCs introduced better effects than those administered via the intratracheal route [13]. We hypothesized that the results of this clinical trial will provide data supporting that UC-MSC administration via the IV route is safe, feasible, and potentially effective in COPD patients.

The dose-escalating evaluation has been conducted in several clinical trials for various diseases, including pulmonary syndromes, using a wide range of UC-MSC doses from 0.5 – 10 million cells/kg via IV administration [15 26 28]. Notably, limited studies have reported the different effects of MSC doses in COPD patients. In fact, a relatively high dose (10 million cells/kg patient body weight) was tested in ARDS patients without any administration-associated AEs or SAEs recorded. However, it is important to note that delivery of a high dose of stem cells might increase the risk of pulmonary embolism and thrombosis regardless of administration route, which was demonstrated previously in animal models and clinical trials [29-31]. Therefore, in this trial, we used the most common dose of MSCs used in numerous studies, which is 1 million cells/kg patient body weight.

In pre-clinical models of elastase-induced emphysema, two doses of MSCs improved anti-inflammatory regulation and lung recover compared to a single dose of MSCs [32]. In clinical trial using BM-MSCs, COPD patients received four infusions showed a reduction in circulating C-reactive protein and in combination with lung volume reduction strategy, two infusions were enough to increase the expression of CD31, an indication of microvascular endothelial cell response [17 25]. Moreover, the effects of autologous MSC administration were reported to be relatively narrow because it was reported the positive effects in patients with type 2 diabetes were observed as early as 1 month post-administration of MSCs and started to diminish after three to six months post-administration [33 34]. Hence, in this current study, we will perform two doses of UC-MSCs with a 3-month intervening interval.

This clinical trial has several advantages. First, this is the first trial using an "off-the-shelf" product (UC-MSCs) for COPD patients. Second, this is the first trial to investigate the therapeutic effects of UC-MSCs as supplementary

products in combination with standard medication treatments according to the GOLD 2019 recommendation. Third, if the potential efficacy can be detected throughout the course of our study, our results (including MSC biological analysis of stem cell characterization, immunoregulation, and metabolism) will strengthen our knowledge and understanding of UC-MSC effects in COPD and provide a fundamental background for treating patients with moderate-to-severe COPD. In the case of no therapeutic effect, our data will also provide important insight into the safety of the treatment and potential alternative approach for MSC therapy of COPD.

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Contributors:

TLN, DMH, and KTN were involved in the design of the study. DMH drafted the manuscripts with critical input from LNT, KTN, AHN, and BNN. LNT, AHN, and BNN contributed to the standard medical treatment checklist and drug for all patients. DMH, LNT and AHN are the grant holder and project leader, respectively. All authors reviewed, edited and approved the final version of the manuscript.

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Conflicts of Interests

None declared.

Patient consent for publication

Not required

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Figure Legend:

Figure 1: Schematic of the study. COPD patients will be screened to enroll in the study. Patients from the control group will be assigned to a patient from the UC-MSC group once they meet all matched criteria based on age (±5 years), gender, and COPD severity classification (GOLD 2019).

Figure 2: Standard COPD medication treatment for both groups according to GOLD 2019 and Vietnam Ministry of Health Guideline. Matched COPD patients will be treated using the same treatment based on their GOPD 2019 classification (Groups A, B, C, and D). Group A (not included in this study): a single bronchodilator will be used and based on the clinical assessments and persistence of the symptoms to continue/stop or replace by another bronchodilator. Group B: Single LAMA or LABA will be initially used. If the symptoms are not reduced, a combination of both LAMA and LABA will be applied. Group C: A single LAMA drug will be used for initial treatment. If exacerbations occur, LAMA and LABA combination will be applied as priority. The LAMA + ICS will be applied in specific cases based on clinical assessment, as the ICS has been reported to have severe side effects on lung inflammation. Group D: Should start the treatment with LAMA. If the patient has CAT>20, LABA and LAMA will be used as initial treatment. LABA + ICS will be used as the initial treatment only when the patient has asthma COPD overlap or the patient's eosinophil level > 300. If exacerbation occurs after the initial treatment, the combination of LAMA, LABA, and ICS should be applied. Additional roflumilast should be used if FEV1 < 50% and the patient has chronic bronchitis. Macrolide should be used if the patient is a former smoker. The red arrow indicates priority treatment.

Table 1: Standard medication treatment for both groups based on GOLD 2019 guidelines and Vietnam Ministry of Health recommendations.

Items	COPD GOLD 2019 Group B	COPD GOLD 2019 Group C	COPD GOLD 2019 Group D
Initial treatment	A long acting bronchodilator (LABA or LAMA)	LAMA	LAMA
Difficulty in breathing (moderate)	LAMA + LABA	LAMA + LABA Or LAMA + ICS	LAMA + LABA ISC/LABA use when: ▪ Asthma COPD overlap. ▪ Eosinophils>300/ul.
Difficulty in breathing (Severe)	LAMA + LABA	LAMA + LABA Or LAMA + ICS	LAMA + LABA + ICS
Name of Drugs use in Standard COPD Medication Treatment for both groups			
SABA	Salbutamol, Terbutaline, Fenoterol		
LABA	Indacaterol, Bambuterol		
SAMA	Ipratropium		
LAMA	Tiotropium		
SABA + SAMA	Ipratropium and salbutamol Ipratropium and fenoterol		
LABA + LAMA	Indacaterol and Glycopyronium Olodaterol and Tiotropium Vilanterol and Umeclidinium		
ICS + LABA	Budesonid and Formoterol Fluticason and Vilanterol Fluticason and Salmeterol		
Antibiotics	Erythromycin Rofumilast[1]		
Long/short-acting Xanthine	Theophyllin/Theostat		

[1]: *Roflumilast was used only when patients' FEV1<50% and had at least 1 admission within 1 year*

Table 2: Study timeline and clinical procedures during the trial. * If the results of the screening phase for UC-MSC groups are within 30 days of UC-MSC administration, they will be automatically considered as the baseline level.

Study Procedure	Prescreening	Screening phase*	Baseline	3 months	6 months	12 months
UC-MSC administration[1]			☑	☑		
Medication treatment[2]			☑	☑	☑	☑
Informed consent		☑				
Inclusion and exclusion criteria		☑				
Demographic information		☑	☑			
Patients' medical reports		☑	☑	☑	☑	☑
Vital signs[3]/physical examination		☑	☑	☑	☑	☑
COPD assessment	☑	☑				
COPD GOLD 2019 classification	☑	☑		☑	☑	☑
Hematology analysis[4]	☑	☑	☑	☑	☑	☑
Infectious disease examination/test[5]	☑	☑	☑			
Blood oxygen saturation/arterial blood gas analysis[6]	☑	☑	☑	☑	☑	☑
Chest CT scan		☑	☑		☑	☑
Chest X-ray		☑	☑		☑	☑
Pulmonary function analysis		☑	☑	☑	☑	☑
Adverse event evaluation			☑	☑	☑	☑
Mortality/complications monitoring			☑	☑	☑	☑

[1]: Applies only for the UC-MSC group at baseline and 3 months.

[2]: Treatment medication applies for all testing groups based on patients' COPD classification according to GOLD 2019 guidelines.

[3]: Vital signs include body temperature, blood pressure, heart rate, respiratory rate, oxygen saturation, and patient body weight.

[4]: Hematological analysis included white blood cell count, platelet count, red blood cell count, hemoglobin, percentage of lymphocytes, neutrophils, monocytes, eosinophils, basophils, C-reactive protein, Pro-BNP, and Troponin-T.

[5]: Infectious diseases include hepatitis, syphilis, HIV, HBV, and tuberculosis.

[6]: Blood gas analysis includes pH, PaO_2, $PaCO_2$, BE, HCO_3-.

Table 3: Release criteria and stem cell quality control. To assess the quality of UC-MSCs for administration, a set of release criteria was defined, which included the following: the positive markers (CD73, CD90, and CD105) must be higher than 95%, the negative markers (CD11b, CD19, CD34, CD45, and HLA-DR) must be less than 2%; the cell viability must be higher than 80% with a normal karyotype; and the cell product must be free from microorganism infections and mycoplasma. Immunoregulatory assays will be performed to assess but not consider released criteria.

Criteria	Testing Method	Released criteria
Positive markers (%) (median, range)		
CD73	Flow cytometry using the Human MSC Analysis Kit (Becton Dickinson,USA)	> 95%
CD90		> 95%
CD105		> 95%
Negative markers (%)		< 2%
Cell viability (%) (mean ± SD)	Trypan Blue staining	> 80%
Microorganism tests	BacT/Alert® 3D microbial detection System (Biomerieux, USA)	Negative
Mycoplasma	MycoAlertTM Plus Mycoplasma Detection Kit (Lonza, Switzerland)	Negative
Endotoxin	Endosafe-PTS (Charles River Laboratories)	≤ 5 EU/kg
Immunoregulatory assay	Flow Cytometry	Not Applicable

Table 4: Proposed experiments design for evaluation the potential therapeutic mechanism of UC-MSCs in the treatment of COPD

Proposed Experiments	Criteria	Cell Type/Method	Expected Outcomes
UC-MSC characterization	MSC marker analysis	UC-MSCs/Flow cytometry	Meet ISCT guideline
	Differentiation potential	UC-MSCs/ *In vitro* differentiation using commercial kits.	Adipogenic, Chondrogenic, and Osteogenic differentiation
	Karyotype	UC-MSCs/ G-banding method	Normal post-expansion
	Growth factor, cytokines secretion	UC-MSCs/ProcartaPlex Immunoassays	Detection of cytokines and growth factors involves in anti-inflammatory and tissue regeneration process.
Metabolic evaluation	Mitochondrial activities	UC-MSCs/Agilent Seahorse XF cell mito stress test	Measurement of mitochondrial activities of UC-MSCs pre-administration
	Glycolysis	UC-MSCs/Agilent Seahorse XF Glycolysis Stress Test	Measurement of glycolysis process of UC-MSC pre-administration
Immunoregulatory Assessment	Lymphocyte Proliferation Assay	UC-MSCs + peripheral mononuclear cells from healthy donors	UC-MSCs inhibit the proliferation rate of lymphocytes in the present of PHA.
		UC-MSCs + peripheral mononuclear cells from COPD patients	UC-MSCs inhibit the proliferation rate of lymphocytes in the present of PHA in a similar manner to healthy donor counterpart
Growth factors and cytokines analysis	UC-MSC secretion profiles under xeno-free and serum-free culture conditions	UC-MSCs/ProcartaPlex Immunoassays	Detection of cytokines and growth factors involves in anti-inflammatory and tissue regeneration process.
	Cytokine profiles from COPD patients' plasma	COPD patients' plasma/ ProcartaPlex Immunoassays	Evaluation of cytokines involves in inflammatory response obtain from COPD patients' plasma before and after UC-MSC administration.
	Interaction between UC-MSCs and COPD patients' lymphocytes	Media obtained from co-culture of UC-MSCs and peripheral mononuclear cells from COPD patients/ ProcartaPlex Immunoassays	Detection of cytokines involves in the anti-inflammatory functions of UC-MSCs.

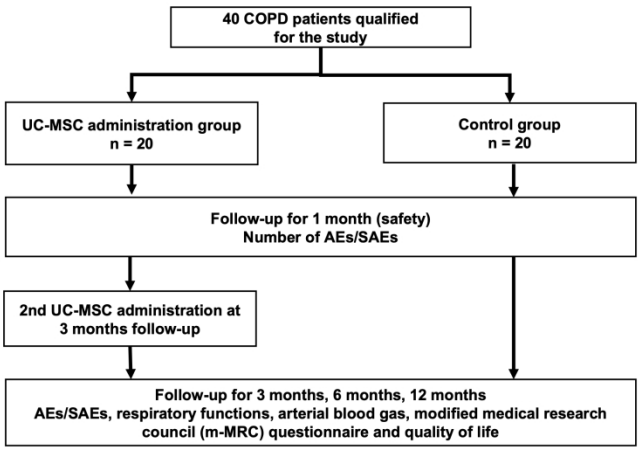

Figure 1

Figure 1: Schematic of the study. COPD patients will be screened to enroll in the study. Patients from the control group will be assigned to a patient from the UC-MSC group once they meet all matched criteria based on age (±5 years), gender, and COPD severity classification (GOLD 2019).

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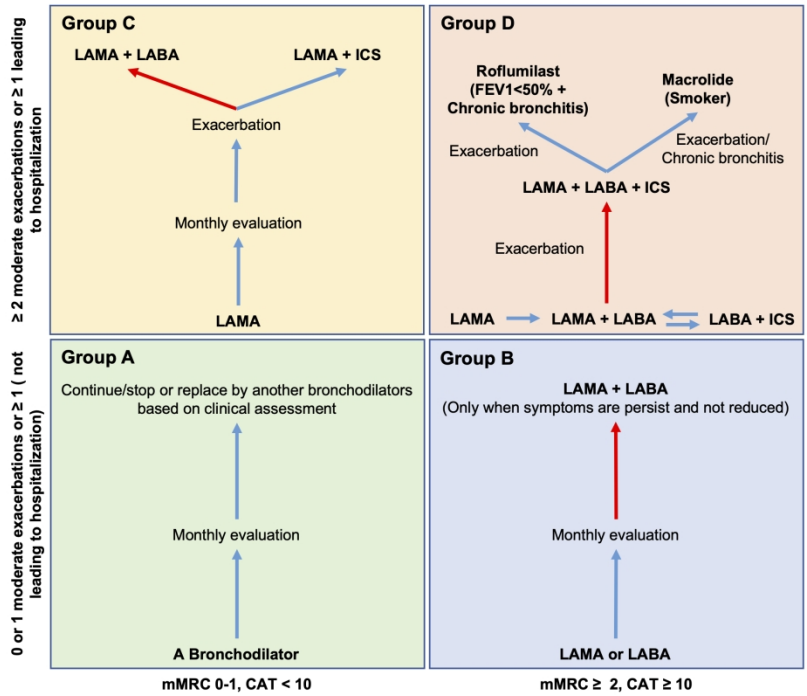

Figure 2:

Figure 2: Standard COPD medication treatment for both groups according to GOLD 2019 and Vietnam Ministry of Health Guideline. Matched COPD patients will be treated using the same treatment based on their GOPD 2019 classification (Groups A, B, C, and D). Group A (not included in this study): a single bronchodilator will be used and based on the clinical assessments and persistence of the symptoms to continue/stop or replace by another bronchodilator. Group B: Single LAMA or LABA will be initially used. If the symptoms are not reduced, a combination of both LAMA and LABA will be applied. Group C: A single LAMA drug will be used for initial treatment. If exacerbations occur, LAMA and LABA combination will be applied as priority. The LAMA + ICS will be applied in specific cases based on clinical assessment, as the ICS has been reported to have severe side effects on lung inflammation. Group D: Should start the treatment with LAMA. If the patient has CAT>20, LABA and LAMA will be used as initial treatment. LABA + ICS will be used as the initial treatment only when the patient has asthma COPD overlap or the patient's eosinophil level > 300. If exacerbation occurs after the initial treatment, the combination of LAMA, LABA, and ICS should be applied. Additional roflumilast should be used if FEV1 < 50% and the patient has chronic bronchitis. Macrolide should be used if the patient is a former smoker. The red arrow indicates priority

treatment.

190x275mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	"n/a"
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	14
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	14

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	14
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4-5
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5, 6
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5, 6

Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6,7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	8, 9
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	8, 9
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8, 9
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9, 10
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	5,6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7,8
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be	"n/a"

		provided in a separate document that is unavailable to those who enrol participants or assign interventions	
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10

Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	10
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	10
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	9, 10
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	10, 11
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5, 6

Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	5,6
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	10
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	"n/a"
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	10
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	14
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	"n/a"
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	"n/a"
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Table 2

BMJ Open

Allogeneic human umbilical cord-derived mesenchymal stem/stromal cells for chronic obstructive pulmonary disease (COPD): study protocol for a matched case-control, phase I/II trial

Journal:	*BMJ Open*
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Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Research methods
Keywords:	Transplant medicine < INTERNAL MEDICINE, RESPIRATORY MEDICINE (see Thoracic Medicine), Chronic airways disease < THORACIC MEDICINE

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Allogeneic human umbilical cord-derived mesenchymal stem/stromal cells for chronic obstructive pulmonary disease (COPD): study protocol for a matched case-control, phase I/II trial.

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Keywords: Umbilical cord-derived Mesenchymal Stem/stromal Cells, Chronic Obstructive Pulmonary Disease (COPD), allogeneic MSC administration, clinical trial.

Word count: 5599 words

Abstract

Introduction

The global prevalence of chronic obstructive pulmonary disease (COPD) is increasing, and it has become a major public health burden worldwide, including in Vietnam. A large body of preclinical and clinical studies supports the safety of mesenchymal stem/stromal cells (MSCs) in the treatment of lung injury, including COPD. The aim of this trial is to investigate the safety and potential therapeutic efficacy of allogeneic administration of umbilical cord-derived MSCs (UC-MSCs) as a supplementary intervention in combination with standard COPD medication treatments in patients with moderate-to-severe COPD based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2019 and Vietnam Ministry of Health's guidelines.

Methods and analysis

This matched case-control phase I/II trial is conducted at Vinmec Times City International Hospital, Hanoi, Vietnam between June 2020 and December 2021. In this study, 40 patients will be enrolled and assigned into two age-, gender- and COPD condition-matched groups, including a UC-MSC group and a control group. Both groups will receive standard COPD medication treatment based on the GOLD 2019 guidelines and the Vietnam Ministry of Health protocol. The UC-MSC group will receive two doses of thawed UC-MSC product with an intervention interval of 3 months. The primary outcome measures will include the incidence of prespecified administration-associated adverse events (AEs) and serious adverse events (SAEs). The efficacy will be evaluated based on the absolute changes in the number of admissions, arterial blood gas analysis, lung function and lung fibrosis via CT scan and chest X-ray. The clinical evaluation will be conducted at baseline and 3, 6, and 12 months post intervention.

Ethics and dissemination

Ethical approval was secured from the Ethical Committee of Vinmec International Hospital (number:166/2019/QĐ-VMEC) and Vietnam Ministry of Health (number:2002/QĐ-BYT). The results will be reported to trial collaborators, publication in peer-reviewed academic journals.

Trial registration number:NCT04433104.

Strengths and Limitations

- This project is the first matched case-control phase I/II study to evaluate the safety and efficacy of allogeneic administration of UC-MSCs as supplementary treatment in combination with standard medication treatments for patients with moderate-to-severe COPD.
- To address the challenge of evaluating the effectiveness of MSC treatment in COPD by using quantitative and qualitative research methods.
- To interlink the treatment effectiveness with stem cell phenotype analysis to broaden our understanding of UC-MSC effects in COPD.
- The limitation of this study is that it was not conducted as a randomized control trial due to the complexity of the process and patient recruitment as well as the challenges of undertaking clinical trials in COPD patients due to the heterogeneity of disease mechanisms and phenotypic expression.

Introduction

Chronic obstructive pulmonary disease (COPD) is described – but not defined – as one of the major chronic lung diseases characterized by persistent and progressive airflow obstruction. It is caused by an elevated chronic pulmonary inflammatory response in the airways and bronchial structure to noxious particles or gases. The pathological hallmarks of the disease include obstructive bronchiolitis, emphysema, and mucus hypersecretion[1]. Despite many medical advancements and technological improvements, our understanding of the pathological mechanisms underlying the progressive and detrimental development of COPD remains incomplete, the definition of the disease is controversial, diagnostic tests are inaccurate and unstandardized, and the treatment is inadequate[2]. A recent report stated that the global prevalence of COPD increased by 44% within the last 20 years, and more than 3.2 million patients died each year from COPD worldwide (accounting for approximately 5% of all deaths globally per year) [3]. In Vietnam, according to the WHO report, 7.1% of males and 1.9% of females aged 40 and above are diagnosed with COPD. Consequently, approximately 25% of hospital beds in respiratory wards are required for COPD patients, resulting in a heavy burden to Vietnamese Medical Infrastructure and reducing patients' health and quality of life [4]. The current pharmacological medications for COPD include the use of inhaled bronchodilator drugs, such as long-acting β agonists (LABAs) and long-acting muscarinic antagonists (LAMAs), the use of inhaled corticosteroids (ICSs) or a combination of these medications. Although it is generally accepted that pharmacological interventions via inhalation would allow the accurate delivery of drugs and increase the clinical benefits, incorrect inhaler technique and a lack of adherence when feeling healthy caused worse dyspnea, impaired health condition, and increased the frequency of exacerbations and hospitalizations in Vietnamese COPD patients [5]. Therefore, identifying novel effective therapies for COPD patients is urgent and important.

Since their first discovery in 1968, mesenchymal stem/stromal cells (MSCs) have been intensively studied because of their therapeutic and regenerative features. The nomenclature of MSCs has been debated recently due to not only the biological features of the MSCs themselves but also the medical abuse of the term "stem cells" inferring direct medical benefit [6]. To standardize the characterization of MSCs and facilitate their therapeutic implications, the International Society for Cellular Therapy (ISCT) has proposed the minimum criteria to define human MSCs [7 8]. In our study, MSCs were defined as mesenchymal stem/stromal cells, which are a class of adult mesenchymal progenitor cells derived from either bone marrow, adipose, or umbilical cord tissue and met the minimum criteria of ISCT. Among various sources of MSCs, human umbilical cord-derived MSCs (hUC-MSCs) are potentially more advanced than their adult counterparts (bone marrow or adipose) for several reasons: (1) ease of collection as it is a noninvasive process, (2) waiving ethical barriers as UC is medical waste discarded at birth, (3) rapid proliferation rate, (4) maintenance of normal karyotype during prolonged culture *in vitro*, and (4) higher paracrine potency than adult tissue-derived MSCs [9]. The therapeutic potential of hUC-MSCs has been proven in clinical studies, especially animal pulmonary disease models, including acute respiratory distress syndrome (ARDS), bronchopulmonary dysplasia (BPD), and COPD. It has been reported that UC-MSCs are effective in reducing lung inflammation and fibrosis processes, preventing secondary infection, decreasing immune system damage, increasing bronchoalveolar fluid clearance, and enhancing the regeneration of alveolar epithelium layers [10-12]. The majority of intravenously

administered MSCs reportedly remain in the lung, especially pulmonary microvessels, which potentially contribute to their beneficial effects in pulmonary disease models [13]. Hence, the safety and therapeutic effects of UC-MSC administration for COPD require further investigation and clarification.

To date, five completed clinical trials have used bone marrow mononuclear cells (BMMCs), bone marrow-derived MSCs (BM-MSCs), and UC-MSCs in COPD [14-19]. Although these clinical trials provided an enormous amount of data supporting the safety of the therapy in the treatment of COPDs, the efficacy of the treatments remained controversial due to several limitations, including trial design, lack of standardization of cell numbers administered to patients, timing of MSC administration, and, most importantly, the lack of a control group in several studies. Moreover, the variations in patient selection based on the severity and stage of COPD could be attributed to the effectiveness of the cell therapy, resulting in caution in data interpretation. Last but not least, the quality of administered MSCs also plays a significant role in the effectiveness of the treatment, i.e., the status of the cells (fresh culture vs. frozen cells), cell sources (from young healthy donors or aging individuals), dosage frequency, etc. Therefore, identification of the potential sources of MSCs (such as UC-MSCs), larger sample size with matched controls, and standardized classification of COPD using international accepted criteria is required to further investigate the safety and efficacy of MSC therapy. Based on preclinical studies and previous promising findings, we designed a matched control phase I/II clinical trial to evaluate the safety and potential efficacy of the intravenous infusion of allogeneic hUC-MSCs in patients with COPD characterized based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2019 criteria[20].

Methods and analysis

Study objectives

The aim of this trial is to evaluate the safety and potential efficacy of allogeneic UC-MSC administration in patients with COPD. There are three specific objectives:

1. Evaluate the safety and potential therapeutic effectiveness of intravenously (IV) administered UC-MSCs in patients with COPD.
2. To prove the hypothesis that IV administration of UC-MSCs can improve lung function and reduce inflammatory responses in the lungs and fibrosis.
3. Explore the potential therapeutic mechanism of UC-MSCs in the treatment of COPD.

Study design and ethics

This matched case-control phase I/II clinical trial was approved by the Ethical Committee of Vinmec International Hospital (number: 166/2019/QĐ-VMEC) and Vietnam Ministry of Health (number: 2002/QĐ-BYT). This study was registered at ClinicalTrials.gov (number NCT04433104). To achieve the aims, a total of 40 patients with COPD will be recruited at the Internal Medicine Department at Vinmec Times City International Hospital, Hanoi, Vietnam, between June 2020 and December 2021. A flowchart of the study design is shown in Figure 1.

Sample size

As a previous study indicated that the FEV_1 (%) of COPD patients was reduced to 35.4±7.1% (6% reduction) after 6 months post-administration, we set this indicator at 18% reduction after 12 months post-administration to calculate the minimum sample size for the proposed study[18 21]. According to the continuous endpoint of two independent sample studies[22], we assumed α was 0.05 and type-II error β was 0.2; thus, the smallest sample size was 40 patients. The calculated sample size was 20 for each group.

Matching strategy:

The patients from the control group will be assigned to a patient from the MSC group once they meet all matched criteria based on age (±5 years), gender, and COPD severity classification (GOLD 2019). Patients from both groups will receive standard COPD medication management according to their COPD severity classification and based on the Vietnam Ministry of Health guideline for COPD treatment, as shown in Figure 2 and Table 1.

A total of 40 patients will be recruited and assigned to the UC-MSC administration group (20 patients) and the control group (20 patients). The trial contains two phases: (1) the first phase will include recruiting and evaluating the first 5 patients from each group to assess the safety of UC-MSC administration after 1 month of follow-up, and (2) the second phase will be initiated after the 1st phase safety report is approved by the Ethical Committee of Vinmec International Hospital and Vietnam Ministry of Health to start recruiting the remaining 15 patients from each group to evaluate both safety and efficacy of the treatment.

Participants

The principal investigators, research and clinician team members are responsible for the study design, patient screening, recruitment, conduct, and perform follow-up assessments in the trial. Participants will be allowed to enroll or withdraw at any time throughout the study. The participants will have all screening and testing costs related to the trials waived except for the costs of COPD medications or drugs. All participants' information will be protected by coding and restricted access using a computer-based system. Participants will be enrolled in the study once they meet all inclusion and exclusion criteria.

Inclusion and exclusion criteria

The diagnostic criteria and severity classification of COPD refers to the criteria established by the COPD 2019 guidelines [20]. Patients will be asked to confirm the COPD conditions and classification from national hospitals and send the results to the administration office prior to enrollment in the trial for prescreening. Patients will be enrolled in the study in compliance with the inclusion and exclusion criteria established by a screening protocol as presented below.

Inclusion Criteria

- Diagnosed with COPD with stage B, C, or D according to GOLD 2019.
- Age between 40-75 years old.
- Both genders.

Exclusion Criteria

- Smoker or less than 6 months of smoking cessation time.
- Asthma and other pulmonary-related diseases and injuries (including lung tuberculosis, restrictive lung disease, idiopathic pulmonary fibrosis, or lung cancer).
- Acute and/or active infection.
- Cancer.
- Patients with complex cardiovascular diseases (including valvular heart disease, cardiomyopathy, arrhythmia, congenital heart disease, hypertrophy syndrome).
- Liver and kidney failure.
- Pregnancy.
- Patients with life expectancy less than 6 months due to concomitant illness.
- Under immunosuppressive treatment within 8 weeks of the first screening visit.
- Patient diagnosed diabetes with $HbA_1C>7\%$.

Recruitment

Patients can only enroll in this study after passing the prescreening process, consultation resolution, and signing the informed consent form.

The recruitment campaign will target three main sources. First, potentially eligible hospitalized patients diagnosed with severe COPD will be approached and asked to participate in the study. Second, physicians will generate lists of patients from the electronic medical system of Vinmec Times City International Hospital with a diagnosis of COPD based on severity classification matching the GOLD 2019 criteria who were discharged within 2 years. Investigators or physicians will contact patients by telephone or mail them a research leaflet and recruitment letter. Third, leaflet and trial recruitment letters will be posted in the Vingroup cooperation internal email system, the official website, and the Facebook public platform of the Vinmec Healthcare system for those diagnosed with COPD GOLD 2019 (B, C, D) at other hospitals. If the patients are interested in this research, we will ask them to send the prescreen results to the administration office.

A multidisciplinary consultation will be held to evaluate the prescreening results from participants to confirm whether these potential participants meet the general diagnostic criteria of COPD, including inclusion and exclusion criteria. The consultation includes physicians and experts from respiratory, radiology, laboratory, and stem cell biology fields. If more than 80% of experts agree on the prescreening results, patients will be viewed as potential participants. The researchers will set an appointment to communicate with the potential candidates about the clinical trial details, including pros and cons of stem cell treatments and sign the written informed consent form prior to assigning patients to either stem cell administration or control groups.

The details of the clinical trial will be explained to patients by investigators or physicians as follows: (1) the study aims and scope, (2) background of COPD and UC-MSC, (3) number of participants, study duration, and classification into either MSC administration or control group, (4) study procedure (including screening, COPD

medication management, follow-up tests), (5) potential discomfort and risks of MSC administration (including prespecified adverse and severe adverse events), (6) expected outcomes of the treatment (primary safety evaluation and potential therapeutic improvement of both MSC administration and COPD medication management according to Vietnam Ministry of Health guideline), (7) protection policy of patients' information and privacy, and (8) voluntary participation (right and responsibility of patients). Patients will only sign written informed consent when all the above items are fully explained and the patients fully understand the protocol. The patients' baseline characteristics will be assessed by the clinicians within 30 days prior to UC-MSC administration for patients in the MSC administration group (Table 2).

Intervention

30 Umbilical cord (UC) samples were obtained from healthy women with an uncomplicated, at term pregnancy who underwent serological testing, including tests for HIV, cytomegalovirus (CMV), Epstein-Barr virus (EBV), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis, and chlamydia, at 38 weeks of pregnancy, as shown in previous study[23]. The UC tissues were collected at delivery and transferred to the Stem Cell Core Facility at the Vinmec Research Institute of Stem Cell and Gene Technology under ISO 14644-1 (certification number: CR61119-1). To generate a UC-MSC line for the current study, a single eligible UC tissue will be processed, isolated and cultured under xeno-free and serum-free conditions as previously described[23]. UC-MSCs will be expanded under these conditions to passage 5 (P5) and cryopreserved in the serum- and xeno-free defined reagent CryoStore® CS10 (Stem Cell Technology, Canada) in liquid nitrogen (gas phase) in an automated Brooks System (Brooks Life Science, USA) for long-term storage. The releasing criteria for UC-MSC products are shown in Table 3.

To prepare UC-MSCs for therapy, aliquots of P5 UC-MSCs will be thawed in a temperature control water bath or incubator on the infusion day. The hUC-MSCs will be washed and suspended in 0.9% normal saline. In addition to inspecting the quality of the UC-MSC product based on the releasing criteria, the staff of the Cell Therapy Department will confirm the viability and quality of the UC-MSC product before the infusion. The cell dose will be calculated based on patients' body weight and cell viability results to obtain the dose of $1x10^6$ viable cells/kg patient body weight prior to transport to the administration ward. Currently, there is no effective treatment for COPD patients. Thus, the intervention group will be given the standard COPD medication management as primary treatment and extra UC-MSC administration, while the control group will receive only the standard COPD treatment (Table 1).

Mode of cell administration (UC-MSC group)

Patients assigned to UC-MSC administration groups will receive two administrations at a dose of 1 million cells/kg patient bodyweight via the IV route with a 3-month intervening interval. On the day of infusion, thawed cells at P5 will be prepared to meet the target administration dose based on the number of viable cells in 10 mL of 0.9% NaCl (Braun, USA) as described above and delivered to the administration ward for infusion at a rate of 20 mL/hour.

Withdrawal

Participant discontinuation may occur upon participant death, severe adverse events (SEAs), other serious disease-limiting involvement, or a direct request from participant to withdraw from the study. Once the participant withdraws from the study, the reasons for the withdrawal and all recorded results will be documented in detail. New participants will not be recruited to replace withdrawn participants.

Adverse events (AEs)

AEs are defined as adverse medical events that occur after the patient signs informed consent until completion of the follow-up period. AEs include abnormal laboratory results, symptoms, or diseases. All AEs will be documented on a written case report form (CRF) and transferred to a research electronic data capture (RedCap) system. Once AEs occur, the physician and clinician in charge will follow the necessary treatment according to the patient's condition and decide whether to suspend clinical research. In terms of severe adverse events (SAEs), the clinician team will follow the first priority to treat principle and be considered an emergency situation. The principal investigators will immediately inform the Ethical Committee and Medical Advisory board of Vinmec Times City International Hospital. Within 24 h, the SAE report should be submitted with full description, while a follow-up SAE report should be submitted to the Ethical Committee of Vinmec Times City International Hospital. Within the 7 days, the SAE report with comments from the Ethical Committee will be submitted to the National Ethical Committee of Vietnam Ministry of Health via post. All participants enrolled in the study will be subjected to an insurance policy that provides ancillary and posttrial medical care in case of injury or death as a result of their participation in the trial.

Outcome evaluation

Primary outcomes (safety)

All required evaluation and laboratory tests with the timeframes are listed in Table 1. To assess safety, the number of AEs or SAEs during stem cell administration (72 h) at 3 months, 6 months, and 12 months after discharge will be evaluated. Body temperature, blood pressure, respiratory rate, heart rate, and SpO_2 will be recorded in real-time before and during MSC administration up to 24 h. Additionally, D-dimer level and patients' blood analysis will be performed at administration and 24 hour post-infusion to monitor the potential thrombotic events as previously reported [24]. As mentioned above, the first phase of this study will involve recruiting five pairs of patients to evaluate the safety prior to initiating the second phase. The safety report of the first phase will cover one month postdischarge and will be submitted to the Ethical Committee of Vinmec Times City International Hospital and the National Ethical Committee of Vietnam Ministry of Health for approval of starting the second phase.

SAEs include death, any critical cardiac event (new ventricular tachycardia, ventricular fibrillation, or asystole, cardiac arrest, cardiac hypertrophy), acute pulmonary distress and embolism, stroke, anaphylactic shock, sepsis, and other conditions that extend the hospital stay. The prespecified AEs include fever, common allergic reactions (rash, edema, erythema, pallor), infection at the administration site, changes in vital signs, and abnormal laboratory test results (including hematological analysis and indicators of liver and kidney functions).

Secondary outcomes (Efficacy)

The efficacy endpoints are as follows: (1) number of admissions and readmissions, (2) general self-efficacy, (3) the number of admissions and unscheduled outpatient visits due to symptoms of COPD, (3) arterial blood gas analysis (including pH, PaO_2, $PaCO_2$, BE, HCO_3-), (4) respiratory functions (FEV_1, FEV_1/FVC, VC, TLC, RV, DLCO, DLNO/DLCO), (5) electrocardiogram, echocardiography, high-resolution chest computed tomography, abdominal ultrasound, abnormality of thyroid and mammary gland, (6) inflammatory response (CRP, Pro-BNP, and Troponin-T) and (7) cytokine analysis from patients' plasma. In addition, the modified medical research council (mMRC) questionnaire and quality of life (Georges Respiratory Questionnaire – SGRQ) will be used to monitor respiratory function improvement. To reveal the therapeutic effects of UC-MSC administration, UC-MSC characterization will be conducted *in vitro*, including MSC marker analysis, metabolic evaluation, immunoregulatory assessment, and cytokine secretion analysis (Table 4).

Follow-up procedure

Follow-up visits will be conducted at 3, 6, and 12 months after hUC-MSC administration. Patients will be asked to come to the hospital to undergo an assessment of their conditions according to the protocol procedure. The safety follow-up will include an extra 1-month follow-up point via telephone and outpatient contact, and patients will only be asked to make an appointment if AEs or SAEs occur.

Data collection

The data accumulated during the trial will be documented in the patients' medical records and the CRF. The quality control officers from Vinmec Times City International Hospital and Vinmec Scientific Research Board independently checked the accuracy and consistency of the CRF data with the original patients' medical records to ensure that the data were accurately entered into the CRF. Once the CRF is checked, within 7 days, all data will be recorded to RedCap software by assigned personnel and crosschecked by principle investigators. There are four data collection points, including baseline and 3 months, 6 months, and 12 months postadministration. The internal auditor of the Vinmec Research Institute of Stem Cell and Gene Technology will review each original research record to confirm the accuracy, consistency, timely records, and meet the standard requirements. Data analysis will be performed using RedCap and statistical analysis software following the statistical analysis strategy (https://redcap.vinmec.com/). The data of this clinical trial will be disseminated with permission from funding bodies and principle investigators through national and international conferences, peer-reviewed publications, and scientific reports. A complete data set will be available upon request after trial completion.

Statistical analysis strategy

Descriptive statistics will be used to illustrate the demographics of the COPD patients. Categorical variables are expressed as proportions, whereas quantitative variables are described as the mean values and their standard deviations or as the medians and their interquartile ranges. The number and type of adverse events/serious adverse events will be compared between the two treatment groups using the Chi square (or Fisher's exact) test. For the intervention and control groups, the indicators (m-MRC, CAT, SGRQ, respiratory functions, and arterial blood gas analysis) at baseline and at 3 months, 6 months, and 12 months will be compared with repeated measures ANOVA.

P-values < 0.05 will be considered statistically significant. The analyses will be performed using Stata version 14 (StataCorp, College Station, TX, USA).

Patient and public involvement

The patients and public were not involved in the design, or conduct, or reporting or dissemination plans of our research.

Ethics and Dissemination

This trial, including the consent form and clinical protocol, was approved by the Ethical Committee of Vinmec International Hospital (number: 166/2019/QĐ-VMEC) and Vietnam Ministry of Health (number: 2002/QĐ-BYT). This study was registered at ClinicalTrials.gov (number NCT04433104). The trial conforms with the Declaration of Helsinki. All participants will provide oral and written informed consent prior to participating in the study. This study will be reported in accordance with the STROBE guidelines for matched case-control trial[25]. We will disseminate the research results through high-quality peer-reviewed open access (via PubMed) journals and presentations at national and international conferences. Finally, an ongoing update of the trial will also be provided and shared annually with our partners in the health system and community agencies according to National Regulation.

Discussion

This study protocol presents the matched-control phase I/II clinical trial evaluating the safety and potential efficacy of allogeneic UC-MSC administration in patients with moderate-to-severe COPD (GOLD 2019). To date, there is no effective treatment available for COPD patients, and pharmacological interventions are hampered by the heterogeneity of disease mechanisms and phenotypic alternation. Therefore, establishing new treatment methods to reduce the devastating effects of COPD is imperative. The body of preclinical studies and human clinical trials suggests that MSC administration emerges as a potential therapeutic approach for COPD because MSCs have been found to be well tolerated and safe in many clinical trials and have proven their effectiveness in animal models. Several clinical trials have been conducted in COPD. Most of these studies were phase 1 safety trials, which uniformly reported no obvious adverse events and serious adverse events as well as no evidence of infusional toxicities during the follow-up period [26]. However, the effectiveness of MSC therapy showed differences among various clinical trials, and a small number of trials have revealed no significant changes in lung function and fibrosis postadministration compared with baseline levels[27]. Therefore, it is important to comprehensively analyze the factors that directly contribute to treatment safety and efficacy.

Human clinical trials were conducted to evaluate the safety and efficacy of MSCs in the treatment of COPD, including five studies using bone marrow-derived cells [14-17 19] and a pilot study using UC-MSCs[18]. The first study supported the safety profile of MSCs administered BMMCs to four COPD patients, although the overall clinical outcomes did not demonstrate the efficacy of the treatment. It is understandable that studies together with the two trials (NCT001110252 and NCT01306513) are phase 1 clinical trials that aimed to evaluate the safety and feasibility of cellular administration in the treatment of COPD. Notably, the NCT001110252 study followed up with patients for

up to 3 years illustrated an overall reduction in the process of COPD pathological development [19]. In a pilot study using UC-MSCs, COPD patients were followed up for 6 months, and no AEs or SAEs were observed throughout the course of the study. Although clinical outcomes such as COPD exacerbations, mMRC score, and CAT were significantly reduced postadministration, pulmonary function parameters remained unchanged compared to baseline [18]. In our current study, we use UC-MSCs as an "off-the-shelf" product for administration, providing flexibility in patient management and standardized products for all treated patients, allowing more accuracy in evaluation. Moreover, by using a matched control design, our study aims to eliminate the variability in COPD conditions between the intervention and control groups to accurately evaluate the safety and efficacy of the treatment. In general, it was confirmed that MSC administration is well tolerated without serious adverse events or administration-associated adverse events and is not associated with significant alterations in spirometry, immune function, cardiovascular activity, or patient quality of life [28].

In both preclinical models of COPD and clinical trials, MSCs regardless of their sources exhibit their therapeutic potential via either anti-inflammatory paracrine actions, reducing the rate of pulmonary fibrosis and/or lung recovery. In rodent models, studies using bone marrow-derived MSCs (BM-MSCs) or adipose-derived MSCs (AD-MSCs) have demonstrated that these cells administered via intravenous injection or intra-tracheal instillation were safe and effective in attenuating airway injury and enhance the recovery of lung functions via reducing airway inflammation and apoptosis [29]. In mice model, administration of UC-MSCs (from Wharton's Jelly) significantly improved the pulmonary function and regeneration in COPD-induced mice compared to the sham group [30]. To date, no comparative study is conducted to address the differences in efficacy introduce by MSCs from different sources. In our previous study, we demonstrated that MSCs from perinatal and adult sources behaved differently even when they were cultured under a standardize culture platform (xeno-free and serum-free) [31]. Therefore, although it seems that MSCs derived from different sources might show similar effects on COPD-induced animal models, we believe that the source of MSCs might play a role in the level of the therapy effectiveness and their mechanism of action might also different, especially when they are exposed to COPD-related microenvironment.

To provide an insight into the mechanism of action of MSC administration in response to COPD conditions, this study aims to evaluate the response of patients' lymphocyte to UC-MSC *in vitro* by co-cultures COPD patient's lymphocytes (before and after UC-MSCs administration at different timepoints) with UC-MSCs to evaluate the potential effect of UC-MSCs on patient's lymphocytes compare to that of healthy donors. Hence, this experiment is not only evaluating the UC-MSC potency but also reveal the potential mechanism of MSC actions in COPD patients. We expect the UC-MSCs would inhibit the proliferation of COPD patient's lymphocytes in a similar manner to that of healthy donor. The culture media of UC-MSC alone, lymphocyte alone, and co-culture of UC-MSCs and lymphocyte will be subjected to cytokines analysis of inflammatory factors such as IL-1β, TNF-α, IL-4, IL-8, IL-10, etc. to identify the release of soluble mediators from UC-MSCs that might involve in reducing lung inflammation. Toward this aim, we speculate the potential mechanism of MSC actions for COPD includes: (1) Reduction of inflammatory reactions at injured airway via either paracrine effects or cell-to-cell contact with immune cells, (2)

reduction of pulmonary fibrosis and airway thickening process, and (3) improvement of parenchymal repair by secretion of wide range of cytokines and growth factors.

The major delivery routes of MSCs in the treatment of pulmonary disease include intraperitoneal (usually in animal models), intranasal or intratracheal, and IV administration. The intratracheal administration of MSCs was performed in children with bronchopulmonary dysplasia in several small uncontrolled studies. However, in terms of COPD, all trials utilized IV administration with the aim of investigating whether systemic administration of MSCs is safe and effective in COPD patients. In fact, the IV administration route is considered a better option compared to intratracheal delivery for several reasons. Previous studies illustrated that IV administration of MSCs was safe and potentially provided therapeutic effects in several lung diseases, including COPD [15 27]. Moreover, a systemic analysis of preclinical studies suggested that IV administration of MSCs introduced better effects than those administered via the intratracheal route [13]. We hypothesized that the results of this clinical trial will provide data supporting that UC-MSC administration via the IV route is safe, feasible, and potentially effective in COPD patients.

The dose-escalating evaluation has been conducted in several clinical trials for various diseases, including pulmonary syndromes, using a wide range of UC-MSC doses from 0.5 – 10 million cells/kg via IV administration [15 27 32]. Notably, limited studies have reported the different effects of MSC doses in COPD patients. In fact, a relatively high dose (10 million cells/kg patient body weight) was tested in ARDS patients without any administration-associated AEs or SAEs recorded. However, it is important to note that delivery of a high dose of stem cells might increase the risk of pulmonary embolism and thrombosis regardless of administration route, which was demonstrated previously in animal models and clinical trials [33-35]. Therefore, in this trial, we used the most common dose of MSCs used in numerous studies, which is 1 million cells/kg patient body weight.

In pre-clinical models of elastase-induced emphysema, two doses of MSCs improved anti-inflammatory regulation and lung recover compared to a single dose of MSCs [36]. In clinical trial using BM-MSCs, COPD patients received four infusions showed a reduction in circulating C-reactive protein and in combination with lung volume reduction strategy, two infusions were enough to increase the expression of CD31, an indication of microvascular endothelial cell response [17 26]. Moreover, the effects of autologous MSC administration were reported to be relatively narrow because it was reported the positive effects in patients with type 2 diabetes were observed as early as 1 month post-administration of MSCs and started to diminish after three to six months post-administration [37 38]. Hence, in this current study, we will perform two doses of UC-MSCs with a 3-month intervening interval.

This clinical trial has several advantages. First, this is the first trial using an "off-the-shelf" product (UC-MSCs) for COPD patients. Second, this is the first trial to investigate the therapeutic effects of UC-MSCs as supplementary products in combination with standard medication treatments according to the GOLD 2019 recommendation. Third, if the potential efficacy can be detected throughout the course of our study, our results (including MSC biological analysis of stem cell characterization, immunoregulation, and metabolism) will strengthen our knowledge and understanding of UC-MSC effects in COPD and provide a fundamental background for treating patients with moderate-to-severe COPD. In the case of no therapeutic effect, our data will also provide important insight into the safety of the treatment and potential alternative approach for MSC therapy of COPD.

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Contributors:

TLN, DMH, and KTN were involved in the design of the study. DMH drafted the manuscripts with critical input from LNT, KTN, AHN, and BNN. LNT, AHN, and BNN contributed to the standard medical treatment checklist and drug for all patients. DMH, LNT and AHN are the grant holder and project leader, respectively. All authors reviewed, edited and approved the final version of the manuscript.

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Conflicts of Interests

None declared.

Patient consent for publication

Not required

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Figure Legend:

Figure 1: Schematic of the study. COPD patients will be screened to enroll in the study. Patients from the control group will be assigned to a patient from the UC-MSC group once they meet all matched criteria based on age (±5 years), gender, and COPD severity classification (GOLD 2019).

Figure 2: Standard COPD medication treatment for both groups according to GOLD 2019 and Vietnam Ministry of Health Guideline. Matched COPD patients will be treated using the same treatment based on their GOPD 2019 classification (Groups A, B, C, and D). Group A (not included in this study): a single bronchodilator will be used and based on the clinical assessments and persistence of the symptoms to continue/stop or replace by another bronchodilator. Group B: Single LAMA or LABA will be initially used. If the symptoms are not reduced, a combination of both LAMA and LABA will be applied. Group C: A single LAMA drug will be used for initial treatment. If exacerbations occur, LAMA and LABA combination will be applied as priority. The LAMA + ICS will be applied in specific cases based on clinical assessment, as the ICS has been reported to have severe side effects on lung inflammation. Group D: Should start the treatment with LAMA. If the patient has CAT>20, LABA and LAMA will be used as initial treatment. LABA + ICS will be used as the initial treatment only when the patient has asthma COPD overlap or the patient's eosinophil level > 300. If exacerbation occurs after the initial treatment, the combination of LAMA, LABA, and ICS should be applied. Additional roflumilast should be used if FEV1 < 50% and the patient has chronic bronchitis. Macrolide should be used if the patient is a former smoker. The red arrow indicates priority treatment.

Table 1: Standard medication treatment for both groups based on GOLD 2019 guidelines and Vietnam Ministry of Health recommendations.

Items	COPD GOLD 2019 Group B	COPD GOLD 2019 Group C	COPD GOLD 2019 Group D
Initial treatment	A long acting bronchodilator (LABA or LAMA)	LAMA	LAMA
Difficulty in breathing (moderate)	LAMA + LABA	LAMA + LABA Or LAMA + ICS	LAMA + LABA ISC/LABA use when: ▪ Asthma COPD overlap. ▪ Eosinophils>300/ul.
Difficulty in breathing (Severe)	LAMA + LABA	LAMA + LABA Or LAMA + ICS	LAMA + LABA + ICS
Name of Drugs use in Standard COPD Medication Treatment for both groups			
SABA	Salbutamol, Terbutaline, Fenoterol		
LABA	Indacaterol, Bambuterol		
SAMA	Ipratropium		
LAMA	Tiotropium		
SABA + SAMA	Ipratropium and salbutamol Ipratropium and fenoterol		
LABA + LAMA	Indacaterol and Glycopyronium Olodaterol and Tiotropium Vilanterol and Umeclidinium		
ICS + LABA	Budesonid and Formoterol Fluticason and Vilanterol Fluticason and Salmeterol		
Antibiotics	Erythromycin Rofumilast[1]		
Long/short-acting Xanthine	Theophyllin/Theostat		

[1]: *Roflumilast was used only when patients' FEV1<50% and had at least 1 admission within 1 year*

Table 2: Study timeline and clinical procedures during the trial. * If the results of the screening phase for UC-MSC groups are within 30 days of UC-MSC administration, they will be automatically considered as the baseline level.

Study Procedure	Prescreening	Screening phase*	Baseline	3 months	6 months	12 months
UC-MSC administration[1]			☑	☑		
Medication treatment[2]			☑	☑	☑	☑
Informed consent		☑				
Inclusion and exclusion criteria		☑				
Demographic information		☑	☑			
Patients' medical reports		☑	☑	☑	☑	☑
Vital signs[3]/physical examination		☑	☑	☑	☑	☑
COPD assessment	☑	☑				
COPD GOLD 2019 classification	☑	☑		☑	☑	☑
Hematology analysis[4]	☑	☑	☑	☑	☑	☑
Infectious disease examination/test[5]	☑	☑	☑			
Blood oxygen saturation/arterial blood gas analysis[6]	☑	☑	☑	☑	☑	☑
Chest CT scan		☑	☑		☑	☑
Chest X-ray		☑	☑		☑	☑
Pulmonary function analysis		☑	☑	☑	☑	☑
Adverse event evaluation			☑	☑	☑	☑
Mortality/complications monitoring			☑	☑	☑	☑

[1]: Applies only for the UC-MSC group at baseline and 3 months.

[2]: Treatment medication applies for all testing groups based on patients' COPD classification according to GOLD 2019 guidelines.

[3]: Vital signs include body temperature, blood pressure, heart rate, respiratory rate, oxygen saturation, and patient body weight.

[4]: Hematological analysis included white blood cell count, platelet count, red blood cell count, hemoglobin, percentage of lymphocytes, neutrophils, monocytes, eosinophils, basophils, C-reactive protein, Pro-BNP, and Troponin-T, and D-dimer.

[5]: Infectious diseases include hepatitis, syphilis, HIV, HBV, and tuberculosis.

[6]: Blood gas analysis includes pH, PaO_2, $PaCO_2$, BE, HCO_3-.

Table 3: Release criteria and stem cell quality control. To assess the quality of UC-MSCs for administration, a set of release criteria was defined, which included the following: the positive markers (CD73, CD90, and CD105) must be higher than 95%, the negative markers (CD11b, CD19, CD34, CD45, and HLA-DR) must be less than 2%; the cell viability must be higher than 80% with a normal karyotype; and the cell product must be free from microorganism infections and mycoplasma. Immunoregulatory assays will be performed to assess but not consider released criteria.

Criteria	Testing Method	Released criteria
Positive markers (%) (median, range)		
CD73	Flow cytometry using the Human MSC Analysis Kit (Becton Dickinson,USA)	> 95%
CD90		> 95%
CD105		> 95%
Negative markers (%)		< 2%
Cell viability (%) (mean ± SD)	Trypan Blue staining	> 80%
Microorganism tests	BacT/Alert® 3D microbial detection System (Biomerieux, USA)	Negative
Mycoplasma	MycoAlertTM Plus Mycoplasma Detection Kit (Lonza, Switzerland)	Negative
Endotoxin	Endosafe-PTS (Charles River Laboratories)	≤ 5 EU/kg
Immunoregulatory assay	Flow Cytometry	Not Applicable

Table 4: Proposed experiments design for evaluation the potential therapeutic mechanism of UC-MSCs in the treatment of COPD

Proposed Experiments	Criteria	Cell Type/Method	Expected Outcomes
UC-MSC characterization	MSC marker analysis	UC-MSCs/Flow cytometry	Meet ISCT guideline
	Differentiation potential	UC-MSCs/ *In vitro* differentiation using commercial kits.	Adipogenic, Chondrogenic, and Osteogenic differentiation
	Karyotype	UC-MSCs/ G-banding method	Normal post-expansion
	Growth factor, cytokines secretion	UC-MSCs/ProcartaPlex Immunoassays	Detection of cytokines and growth factors involves in anti-inflammatory and tissue regeneration process.
Metabolic evaluation	Mitochondrial activities	UC-MSCs/Agilent Seahorse XF cell mito stress test	Measurement of mitochondrial activities of UC-MSCs pre-administration
	Glycolysis	UC-MSCs/Agilent Seahorse XF Glycolysis Stress Test	Measurement of glycolysis process of UC-MSC pre-administration
Immunoregulatory Assessment	Lymphocyte Proliferation Assay	UC-MSCs + peripheral mononuclear cells from healthy donors	UC-MSCs inhibit the proliferation rate of lymphocytes in the present of PHA.
		UC-MSCs + peripheral mononuclear cells from COPD patients	UC-MSCs inhibit the proliferation rate of lymphocytes in the present of PHA in a similar manner to healthy donor counterpart
Growth factors and cytokines analysis	UC-MSC secretion profiles under xeno-free and serum-free culture conditions	UC-MSCs/ProcartaPlex Immunoassays	Detection of cytokines and growth factors involves in anti-inflammatory and tissue regeneration process.
	Cytokine profiles from COPD patients' plasma	COPD patients' plasma/ ProcartaPlex Immunoassays	Evaluation of cytokines involves in inflammatory response obtain from COPD patients' plasma before and after UC-MSC administration.
	Interaction between UC-MSCs and COPD patients' lymphocytes	Media obtained from co-culture of UC-MSCs and peripheral mononuclear cells from COPD patients/ ProcartaPlex Immunoassays	Detection of cytokines involves in the anti-inflammatory functions of UC-MSCs.

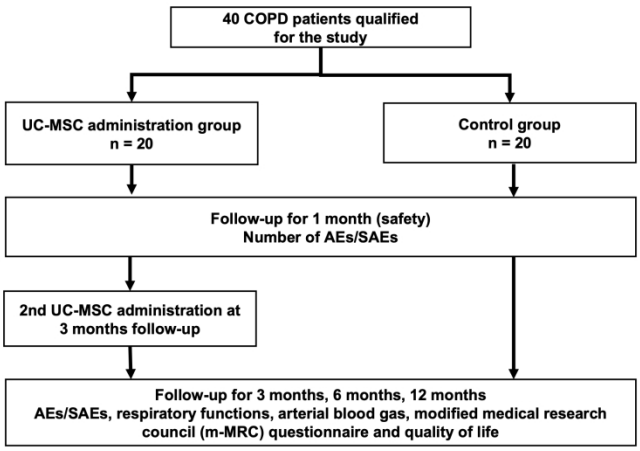

Figure 1

Figure 1: Schematic of the study. COPD patients will be screened to enroll in the study. Patients from the control group will be assigned to a patient from the UC-MSC group once they meet all matched criteria based on age (±5 years), gender, and COPD severity classification (GOLD 2019).

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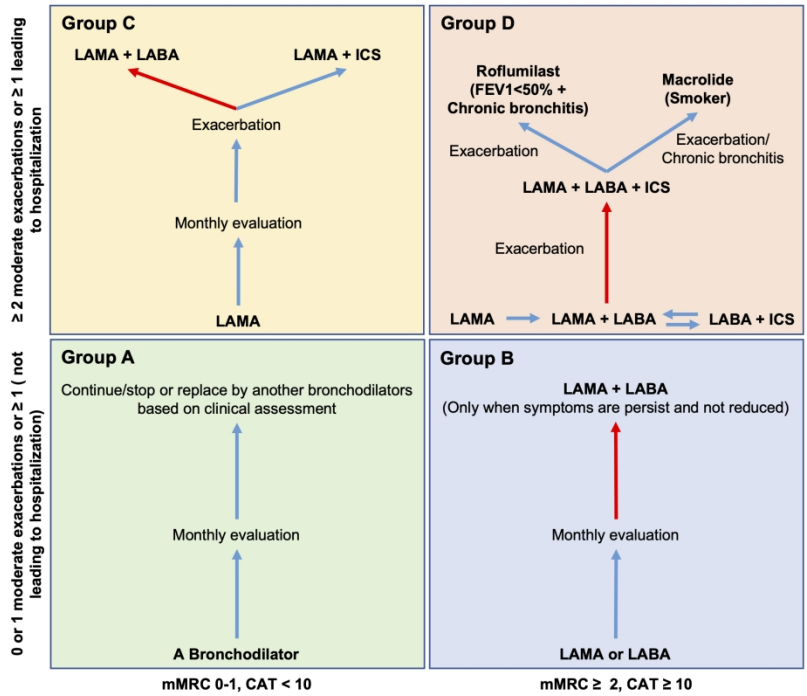

Figure 2:

Figure 2: Standard COPD medication treatment for both groups according to GOLD 2019 and Vietnam Ministry of Health Guideline. Matched COPD patients will be treated using the same treatment based on their GOPD 2019 classification (Groups A, B, C, and D). Group A (not included in this study): a single bronchodilator will be used and based on the clinical assessments and persistence of the symptoms to continue/stop or replace by another bronchodilator. Group B: Single LAMA or LABA will be initially used. If the symptoms are not reduced, a combination of both LAMA and LABA will be applied. Group C: A single LAMA drug will be used for initial treatment. If exacerbations occur, LAMA and LABA combination will be applied as priority. The LAMA + ICS will be applied in specific cases based on clinical assessment, as the ICS has been reported to have severe side effects on lung inflammation. Group D: Should start the treatment with LAMA. If the patient has CAT>20, LABA and LAMA will be used as initial treatment. LABA + ICS will be used as the initial treatment only when the patient has asthma COPD overlap or the patient's eosinophil level > 300. If exacerbation occurs after the initial treatment, the combination of LAMA, LABA, and ICS should be applied. Additional roflumilast should be used if FEV1 < 50% and the patient has chronic bronchitis. Macrolide should be used if the patient is a former smoker. The red arrow indicates priority

treatment.

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	"n/a"
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	14
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	14

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	14
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4-5
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5, 6
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5, 6

Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6,7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	8, 9
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	8, 9
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8, 9
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9, 10
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	5,6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7,8
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be	"n/a"

		provided in a separate document that is unavailable to those who enrol participants or assign interventions	
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10

Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	10
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	10
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	9, 10
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	10, 11
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5, 6

Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	5,6
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	10
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	"n/a"
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	10
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	14
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	"n/a"
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	"n/a"
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Table 2