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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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3 **Allogeneic human umbilical cord-derived mesenchymal stem/stromal cells for chronic obstructive pulmonary**
4 **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/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).

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 hypersecretion¹. 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 inadequate². 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 life⁴. 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 patients⁵. 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 benefit⁶. 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 MSCs⁹. 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 layers¹⁰⁻¹². The majority of intravenously

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3 administered MSCs reportedly remain in the lung, especially pulmonary microvessels, which potentially contribute to
4 their beneficial effects in pulmonary disease models¹³. Hence, the safety and therapeutic effects of UC-MSC
5 administration for COPD require further investigation and clarification.
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8 To date, five completed clinical trials have used bone marrow mononuclear cells (BMMCs), bone marrow-
9 derived MSCs (BM-MSCs), and UC-MSCs in COPD¹⁴⁻¹⁹. Although these clinical trials provided an enormous amount
10 of data supporting the safety of the therapy in the treatment of COPDs, the efficacy of the treatments remained
11 controversial due to several limitations, including trial design, lack of standardization of cell numbers administered to
12 patients, timing of MSC administration, and, most importantly, the lack of a control group in several studies. Moreover,
13 the variations in patient selection based on the severity and stage of COPD could be attributed to the effectiveness of
14 the cell therapy, resulting in caution in data interpretation. Last but not least, the quality of administered MSCs also
15 plays a significant role in the effectiveness of the treatment, i.e., the status of the cells (fresh culture vs. frozen cells),
16 cell sources (from young healthy donors or aging individuals), dosage frequency, etc. Therefore, identification of the
17 potential sources of MSCs (such as UC-MSCs), larger sample size with matched controls, and standardized
18 classification of COPD using international accepted criteria is required to further investigate the safety and efficacy
19 of MSC therapy. Based on the large body of preclinical studies and previous promising findings, we designed a
20 matched control phase I/II clinical trial to evaluate the safety and efficacy of the intravenous infusion of allogeneic
21 hUC-MSCs in patients with COPD characterized based on the Global Initiative for Chronic Obstructive Lung Disease
22 (GOLD) 2019 criteria²⁰.
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30 **Methods and analysis**

31 *Study objectives*

32 The aim of this trial is to evaluate the safety and efficacy of allogeneic UC-MSC administration in patients with COPD.
33 There are two specific objectives:
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- 38 1. Evaluate the safety and potential therapeutic effectiveness of intravenously (IV) administered UC-MSCs in
39 patients with COPD.
- 40 2. To prove the hypothesis that IV administration of UC-MSCs can improve lung function and reduce
41 inflammatory responses in the lungs and fibrosis.
- 42 3. Explore the potential therapeutic mechanism of UC-MSCs in the treatment of COPD.
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46 *Study design and ethics*

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

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3 As a previous study indicated that the mMRC score of COPD patients ranged from 18% to 60% reduction, we
4 set this rate at 60% reduction to calculate the minimum sample size for the proposed study^{18 21}. According to the
5 dichotomous endpoint of two independent sample studies²², we assumed α was 0.05 and type-II error β was 0.2; thus,
6 the smallest sample size was 40 patients. The calculated sample size was 20 for each group.
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9 ***Matching strategy:***

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11 The patients from the control group will be assigned to a patient from the MSC group once they meet all
12 matched criteria based on age (± 5 years), gender, and COPD severity classification (GOLD 2019). Patients from both
13 groups will receive standard COPD medication management according to their COPD severity classification and based
14 on the Vietnam Ministry of Health guideline for COPD treatment, as shown in Figure 2 and Table 3.
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18 A total of 40 patients will be recruited and assigned to the UC-MSA administration group (20 patients) and the
19 control group (20 patients). The trial contains two phases: (1) the first phase will include recruiting and evaluating the
20 first 5 patients from each group to assess the safety of UC-MSA administration after 1 month of follow-up, and (2)
21 the second phase will be initiated after the 1st phase safety report is approved by the Ethical Committee of Vinmec
22 International Hospital and Vietnam Ministry of Health to start recruiting the remaining 15 patients from each group
23 to evaluate both safety and efficacy of the treatment.
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27 ***Participants***

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

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40 The diagnostic criteria and severity classification of COPD refers to the criteria established by the COPD 2019
41 guidelines²⁰. Patients will be asked to confirm the COPD conditions and classification from national hospitals and
42 send the results to the administration office prior to enrollment in the trial for prescreening. Patients will be enrolled
43 in the study in compliance with the inclusion and exclusion criteria established by a screening protocol as presented
44 below.
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48 ***Inclusion Criteria***

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

52 ***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

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

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16 A single umbilical cord (UC) sample will obtained from healthy women with an uncomplicated, at term
17 pregnancy who underwent serological testing, including tests for HIV, cytomegalovirus (CMV), Epstein-Barr virus
18 (EBV), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis, and chlamydia, at 38
19 weeks of pregnancy. The eligible UC tissue will be collected at delivery and transferred to the Stem Cell Core Facility
20 at the Vinmec Research Institute of Stem Cell and Gene Technology under ISO 14644-1 (certification number:
21 CR61119-1). The UC tissue will be processed, and the UC-MSCs will be isolated and cultured under xeno-free and
22 serum-free conditions as previously described. UC-MSCs will be expanded under these conditions to passage 5 (P5)
23 and cryopreserved in the serum- and xeno-free defined reagent CryoStore® CS10 (Stem Cell Technology, Canada) in
24 liquid nitrogen (gas phase) in an automated Brooks System (Brooks Life Science, USA) for long-term storage. The
25 releasing criteria for UC-MSC products are shown in Table 2.
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31 To prepare UC-MSCs for therapy, aliquots of P5 UC-MSCs will be thawed in a temperature control water bath
32 or incubator on the infusion day. The hUC-MSCs will be washed and suspended in 0.9% normal saline. In addition to
33 inspecting the quality of the UC-MSC product based on the releasing criteria, the staff of the Cell Therapy Department
34 will confirm the viability and quality of the UC-MSC product before the infusion. The cell dose will be calculated
35 based on patients' body weight and cell viability results to obtain the dose of 1×10^6 viable cells/kg patient body weight
36 prior to transport to the administration ward. Currently, there is no effective treatment for COPD patients. Thus, the
37 intervention group will be given the standard COPD medication management as primary treatment and extra UC-
38 MSC administration, while the control group will receive only the standard COPD treatment (Table 3).
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43 ***Mode of cell administration (UC-MSC group)***

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

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

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

27 ***Primary outcomes (safety)***

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29 All required evaluation and laboratory tests with the timeframes are listed in Table 1. To assess safety, the
30 number of AEs or SAEs during stem cell administration (72 h) at 3 months, 6 months, and 12 months after discharge
31 will be evaluated. Body temperature, blood pressure, respiratory rate, heart rate, and SpO₂ will be recorded before and
32 during MSC administration up to 24 h. As mentioned above, the first phase of this study will involve recruiting five
33 pairs of patients to evaluate the safety prior to initiating the second phase. The safety report of the first phase will
34 cover one month postdischarge and will be submitted to the Ethical Committee of Vinmec Times City International
35 Hospital and the National Ethical Committee of Vietnam Ministry of Health for approval of starting the second phase.
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41 SAEs include death, any critical cardiac event (new ventricular tachycardia, ventricular fibrillation, or asystole,
42 cardiac arrest, cardiac hypertrophy), acute pulmonary distress and embolism, stroke, anaphylactic shock, sepsis, and
43 other conditions that extend the hospital stay. The prespecified AEs include fever, common allergic reactions (rash,
44 edema, erythema, pallor), infection at the administration site, changes in vital signs, and abnormal laboratory test
45 results (including hematological analysis and indicators of liver and kidney functions).
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49 ***Secondary outcomes (Efficacy)***

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51 The efficacy endpoints are as follows: (1) number of admissions and readmissions, (2) general self-efficacy,
52 (3) the number of admissions and unscheduled outpatient visits due to symptoms of COPD, (3) arterial blood gas
53 analysis (including pH, PaO₂, PaCO₂, BE, HCO₃⁻), (4) respiratory functions (FEV₁, FEV₁/FVC, VC, TLC, RV,
54 DLCO, DLNO/DLCO), (5) electrocardiogram, echocardiography, high-resolution chest computed tomography,
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3 abdominal ultrasound, abnormality of thyroid and mammary gland, (6) inflammatory response (CRP, Pro-BNP, and
4 Troponin-T) and (7) cytokine analysis from patients' plasma. In addition, the modified medical research council
5 (mMRC) questionnaire and quality of life (Georges Respiratory Questionnaire – SGRQ) will be used to monitor
6 respiratory function improvement. To reveal the therapeutic effects of UC-MSC administration, UC-MSC
7 characterization will be conducted *in vitro*, including MSC marker analysis, metabolic evaluation, immunoregulatory
8 assessment, and cytokine secretion analysis.
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11 ***Follow-up procedure***

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

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

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

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

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11 This study protocol presents the matched-control phase I/II clinical trial evaluating the safety and potential
12 efficacy of allogeneic UC-MSC administration in patients with moderate-to-severe COPD (GOLD 2019). To date,
13 there is no effective treatment available for COPD patients, and pharmacological interventions are hampered by the
14 heterogeneity of disease mechanisms and phenotypic alternation. Therefore, establishing new treatment methods to
15 reduce the devastating effects of COPD is imperative. The body of preclinical studies and human clinical trials
16 suggests that MSC administration emerges as a potential therapeutic approach for COPD because MSCs have been
17 found to be well tolerated and safe in many clinical trials and have proven their effectiveness in animal models.
18 However, the effectiveness of MSC therapy showed differences among various clinical trials, and a small number of
19 trials have revealed no significant changes in lung function and fibrosis postadministration compared with baseline
20 levels²³. Therefore, it is important to comprehensively analyze the factors that directly contribute to treatment efficacy.
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24 Human clinical trials were conducted to evaluate the safety and efficacy of MSCs in the treatment of COPD,
25 including five studies using bone marrow-derived cells^{14-17 19} and a pilot study using UC-MSCs¹⁸. The first study
26 supported the safety profile of MSCs administered BMSCs to four COPD patients, although the overall clinical
27 outcomes did not demonstrate the efficacy of the treatment. It is understandable that studies together with the two
28 trials (NCT001110252 and NCT01306513) are phase 1 clinical trials that aimed to evaluate the safety and feasibility
29 of cellular administration in the treatment of COPD. Notably, the NCT001110252 study followed up with patients for
30 up to 3 years illustrated an overall reduction in the process of COPD pathological development¹⁹. In a pilot study
31 using UC-MSCs, COPD patients were followed up for 6 months, and no AEs or SAEs were observed throughout the
32 course of the study. Although clinical outcomes such as COPD exacerbations, mMRC score, and CAT were
33 significantly reduced postadministration, pulmonary function parameters remained unchanged compared to baseline
34¹⁸. In our current study, we use UC-MSCs as an “off-the-shelf” product for administration, providing flexibility in
35 patient management and standardized products for all treated patients, allowing more accuracy in evaluation.
36 Moreover, by using a matched control design, our study aims to eliminate the variability in COPD conditions between
37 the intervention and control groups to accurately evaluate the safety and efficacy of the treatment. In general, it was
38 confirmed that MSC administration is well tolerated without serious adverse events or administration-associated
39 adverse events and is not associated with significant alterations in spirometry, immune function, cardiovascular
40 activity, or patient quality of life²⁴.
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52 The major delivery routes of MSCs in the treatment of pulmonary disease include intraperitoneal (usually in
53 animal models), intranasal or intratracheal, and IV administration. The intratracheal administration of MSCs was
54 performed in children with bronchopulmonary dysplasia in several small uncontrolled studies. However, in terms of
55 COPD, all trials utilized IV administration with the aim of investigating whether systemic administration of MSCs is
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3 safe and effective in COPD patients. In fact, the IV administration route is considered a better option compared to
4 intratracheal delivery for several reasons. Previous studies illustrated that IV administration of MSCs was safe and
5 potentially provided therapeutic effects in several lung diseases, including COPD^{15,23}. Moreover, a systemic analysis
6 of preclinical studies suggested that IV administration of MSCs introduced better effects than those administered via
7 the intratracheal route¹³. We hypothesized that the results of this clinical trial will provide data supporting that UC-
8 MSC administration via the IV route is safe, feasible, and potentially effective in COPD patients.
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12 The dose-escalating evaluation has been conducted in several clinical trials for various diseases, including
13 pulmonary syndromes, using a wide range of UC-MSC doses from 0.5 – 10 million cells/kg via IV administration¹⁵
14^{23,25}. Notably, limited studies have reported the different effects of MSC doses in COPD patients. In fact, a relatively
15 high dose (10 million cells/kg patient body weight) was tested in ARDS patients without any administration-associated
16 AEs or SAEs recorded. However, it is important to note that delivery of a high dose of stem cells might increase the
17 risk of pulmonary embolism and thrombosis regardless of administration route, which was demonstrated previously
18 in animal models and clinical trials²⁶⁻²⁸. Therefore, in this trial, we used the most common dose of MSCs used in
19 numerous studies, which is 1 million cells/kg patient body weight.
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23 This clinical trial has several advantages. First, this is the first trial using an “off-the-shelf” product (UC-MSCs)
24 for COPD patients. Second, this is the first trial to investigate the therapeutic effects of UC-MSCs as supplementary
25 products in combination with standard medication treatments according to the GOLD 2019 recommendation. Third,
26 if the potential efficacy can be detected throughout the course of our study, our results (including MSC biological
27 analysis of stem cell characterization, immunoregulation, and metabolism) will strengthen our knowledge and
28 understanding of UC-MSC effects in COPD and provide a fundamental background for treating patients with
29 moderate-to-severe COPD. In the case of no therapeutic effect, our data will also provide important insight into the
30 safety of the treatment and potential alternative approach for MSC therapy of COPD.
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35
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39 experiment set up.
40
41

42 Contributors:

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44 TLN, DMH, and KTN were involved in the design of the study. DMH drafted the manuscripts with critical input from
45 LNT, KTN, AHN, and BNN. LNT, AHN, and BNN contributed to the standard medical treatment checklist and drug
46 for all patients. DMH, LNT and AHN are the grant holder and project leader, respectively. All authors reviewed,
47 edited and approved the final version of the manuscript.
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52
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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 GOLD 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			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		
Medication treatment ²			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Informed consent		<input checked="" type="checkbox"/>				
Inclusion and exclusion criteria		<input checked="" type="checkbox"/>				
Demographic information		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			
Patients' medical reports		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Vital signs ³ /physical examination		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
COPD assessment	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>				
COPD GOLD 2019 classification	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Hematology analysis ⁴	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Infectious disease examination/test ⁵	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			
Blood oxygen saturation/arterial blood gas analysis ⁶	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Chest CT scan		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Chest X-ray		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Pulmonary function analysis		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Adverse event evaluation			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Mortality/complications monitoring			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

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.

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

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

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9: Infectious diseases include hepatitis, syphilis, HIV, HBV, and tuberculosis.

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11: Blood gas analysis includes pH, PaO₂, PaCO₂, BE, HCO₃⁻.

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For peer review only

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)		
<i>CD73</i>		> 95%
<i>CD90</i>	Flow cytometry using the Human MSC	> 95%
<i>CD105</i>	Analysis Kit (Becton Dickinson, USA)	> 95%
Negative markers (%)		< 2%
Cell viability (%) (mean \pm SD)	Trypan Blue staining	> 80%
Microorganism tests	BacT/Alert® 3D microbial detection System (Biomerieux, USA)	Negative
Mycoplasma	MycoAlert™ Plus Mycoplasma Detection Kit (Lonza, Switzerland)	Negative
Endotoxin	Endosafe-PTS (Charles River Laboratories)	\leq 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: <ul style="list-style-type: none"> ▪ 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.

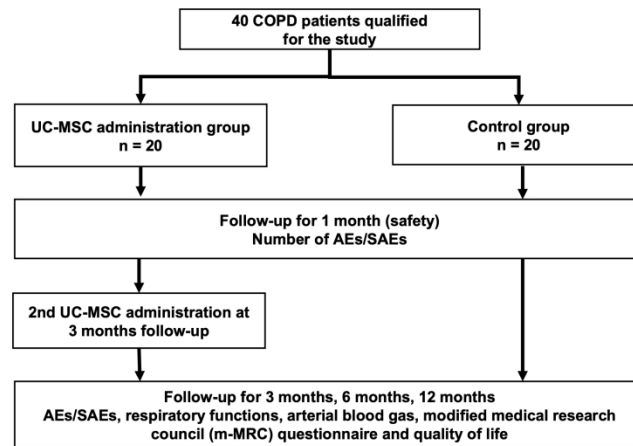


Figure 1

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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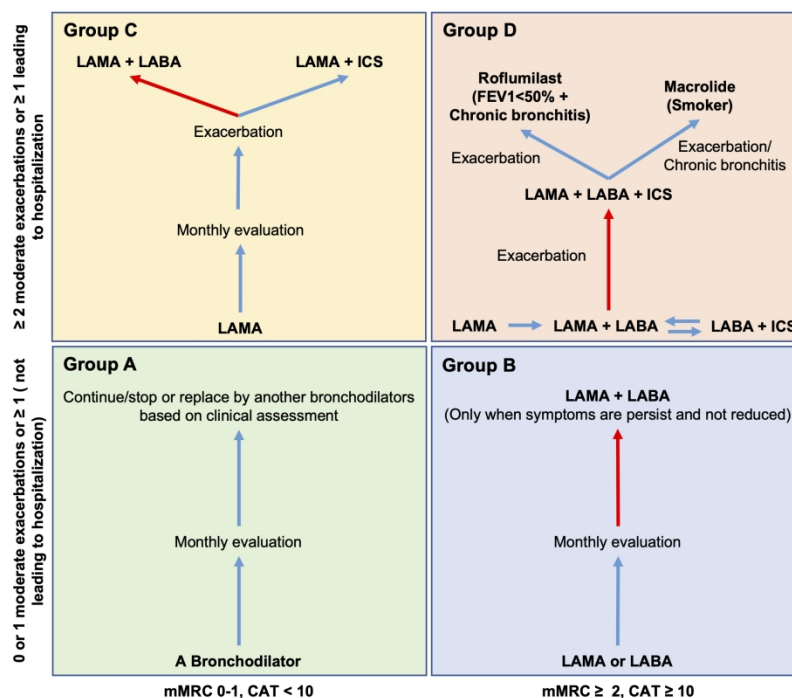


Figure 2:

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 GOLD 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

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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 SPIRIT reporting 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

1	Roles and	#5b	Name and contact information for the trial sponsor	14
2	responsibilities:			
3	sponsor contact			
4	information			
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8	Roles and	#5c	Role of study sponsor and funders, if any, in study design;	14
9	responsibilities:		collection, management, analysis, and interpretation of data;	
10	sponsor and funder		writing of the report; and the decision to submit the report for	
11			publication, including whether they will have ultimate authority	
12			over any of these activities	
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15				
16	Roles and	#5d	Composition, roles, and responsibilities of the coordinating	14
17	responsibilities:		centre, steering committee, endpoint adjudication committee,	
18	committees		data management team, and other individuals or groups	
19			overseeing the trial, if applicable (see Item 21a for data	
20			monitoring committee)	
21				
22				
23				
24	Introduction			
25				
26				
27	Background and	#6a	Description of research question and justification for undertaking	4
28	rationale		the trial, including summary of relevant studies (published and	
29			unpublished) examining benefits and harms for each intervention	
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32	Background and	#6b	Explanation for choice of comparators	4-5
33	rationale: choice of			
34	comparators			
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37	Objectives	#7	Specific objectives or hypotheses	5
38				
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40	Trial design	#8	Description of trial design including type of trial (eg, parallel	5, 6
41			group, crossover, factorial, single group), allocation ratio, and	
42			framework (eg, superiority, equivalence, non-inferiority,	
43			exploratory)	
44				
45				
46	Methods:			
47	Participants,			
48	interventions, and			
49	outcomes			
50				
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52				
53	Study setting	#9	Description of study settings (eg, community clinic, academic	5, 6
54			hospital) and list of countries where data will be collected.	
55			Reference to where list of study sites can be obtained	
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1	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
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6	Interventions:	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
7	description			
8				
9				
10	Interventions:	#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
11	modifications			
12				
13				
14				
15	Interventions:	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	8, 9
16	adherence			
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19				
20	Interventions:	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8, 9
21	concomitant care			
22				
23				
24	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
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34	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
35				
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40	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
41				
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44				
45	Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7,8
46				
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49	Methods: Assignment			
50	of interventions (for			
51	controlled trials)			
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54	Allocation: sequence	#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"
55	generation			
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provided in a separate document that is unavailable to those who enrol participants or assign interventions

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4	Allocation	#16b	Mechanism of implementing the allocation sequence (eg, central
5	concealment		telephone; sequentially numbered, opaque, sealed envelopes),
6			describing any steps to conceal the sequence until interventions
7	mechanism		are assigned
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11	Allocation:	#16c	Who will generate the allocation sequence, who will enrol
12	implementation		participants, and who will assign participants to interventions
13			
14	Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial
15			participants, care providers, outcome assessors, data analysts),
16			and how
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18			
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20	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is permissible,
21	emergency unblinding		and procedure for revealing a participant's allocated intervention
22			during the trial
23			
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25	Methods: Data		
26	collection,		
27	management, and		
28	analysis		
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32	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and
33			other trial data, including any related processes to promote data
34			quality (eg, duplicate measurements, training of assessors) and a
35			description of study instruments (eg, questionnaires, laboratory
36			tests) along with their reliability and validity, if known.
37			Reference to where data collection forms can be found, if not in
38			the protocol
39			
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43	Data collection plan:	#18b	Plans to promote participant retention and complete follow-up,
44	retention		including list of any outcome data to be collected for participants
45			who discontinue or deviate from intervention protocols
46			
47			
48	Data management	#19	Plans for data entry, coding, security, and storage, including any
49			related processes to promote data quality (eg, double data entry;
50			range checks for data values). Reference to where details of data
51			management procedures can be found, if not in the protocol
52			
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54			
55	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary
56			outcomes. Reference to where other details of the statistical
57			analysis plan can be found, if not in the protocol
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1	Statistics: additional	#20b	Methods for any additional analyses (eg, subgroup and adjusted	10
2	analyses		analyses)	
3				
4	Statistics: analysis	#20c	Definition of analysis population relating to protocol non-	10
5	population and missing		adherence (eg, as randomised analysis), and any statistical	
6	data		methods to handle missing data (eg, multiple imputation)	
7				
8				
9				
10	Methods: Monitoring			
11				
12	Data monitoring:	#21a	Composition of data monitoring committee (DMC); summary of	10
13	formal committee		its role and reporting structure; statement of whether it is	
14			independent from the sponsor and competing interests; and	
15			reference to where further details about its charter can be found,	
16			if not in the protocol. Alternatively, an explanation of why a	
17			DMC is not needed	
18				
19				
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21				
22	Data monitoring:	#21b	Description of any interim analyses and stopping guidelines,	10
23	interim analysis		including who will have access to these interim results and make	
24			the final decision to terminate the trial	
25				
26				
27	Harms	#22	Plans for collecting, assessing, reporting, and managing solicited	9
28			and spontaneously reported adverse events and other unintended	
29			effects of trial interventions or trial conduct	
30				
31				
32				
33	Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and	9, 10
34			whether the process will be independent from investigators and	
35			the sponsor	
36				
37				
38	Ethics and			
39	dissemination			
40				
41				
42	Research ethics	#24	Plans for seeking research ethics committee / institutional review	10
43	approval		board (REC / IRB) approval	
44				
45				
46	Protocol amendments	#25	Plans for communicating important protocol modifications (eg,	10, 11
47			changes to eligibility criteria, outcomes, analyses) to relevant	
48			parties (eg, investigators, REC / IRBs, trial participants, trial	
49			registries, journals, regulators)	
50				
51				
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53	Consent or assent	#26a	Who will obtain informed consent or assent from potential trial	5, 6
54			participants or authorised surrogates, and how (see Item 32)	
55				
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1	Consent or assent:	#26b	Additional consent provisions for collection and use of	5,6
2	ancillary studies		participant data and biological specimens in ancillary studies, if	
3			applicable	
4				
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6	Confidentiality	#27	How personal information about potential and enrolled	10
7			participants will be collected, shared, and maintained in order to	
8			protect confidentiality before, during, and after the trial	
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11	Declaration of interests	#28	Financial and other competing interests for principal investigators	14
12			for the overall trial and each study site	
13				
14				
15	Data access	#29	Statement of who will have access to the final trial dataset, and	10
16			disclosure of contractual agreements that limit such access for	
17			investigators	
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20	Ancillary and post trial	#30	Provisions, if any, for ancillary and post-trial care, and for	"n/a"
21	care		compensation to those who suffer harm from trial participation	
22				
23				
24	Dissemination policy:	#31a	Plans for investigators and sponsor to communicate trial results	10
25	trial results		to participants, healthcare professionals, the public, and other	
26			relevant groups (eg, via publication, reporting in results	
27			databases, or other data sharing arrangements), including any	
28			publication restrictions	
29				
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32				
33	Dissemination policy:	#31b	Authorship eligibility guidelines and any intended use of	14
34	authorship		professional writers	
35				
36				
37	Dissemination policy:	#31c	Plans, if any, for granting public access to the full protocol,	"n/a"
38	reproducible research		participant-level dataset, and statistical code	
39				
40				
41	Appendices			
42				
43	Informed consent	#32	Model consent form and other related documentation given to	"n/a"
44	materials		participants and authorised surrogates	
45				
46				
47	Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of	Table 2
48			biological specimens for genetic or molecular analysis in the	
49			current trial and for future use in ancillary studies, if applicable	
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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

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Primary Subject Heading:	Respiratory medicine
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Keywords:	Transplant medicine < INTERNAL MEDICINE, RESPIRATORY MEDICINE (see Thoracic Medicine), Chronic airways disease < THORACIC MEDICINE

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3 **Allogeneic human umbilical cord-derived mesenchymal stem/stromal cells for chronic obstructive pulmonary**
4 **disease (COPD): study protocol for a matched case-control, phase I/II trial.**
5

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9

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18
19 **Keywords:** Umbilical cord-derived Mesenchymal Stem/stromal Cells, Chronic Obstructive Pulmonary Disease
20 (COPD), allogeneic MSC administration, clinical trial.
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3 **1 Abstract**

4
5 **2 Introduction**

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

10 **Methods and analysis**

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

21 **Ethics and dissemination**

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

25 **Trial registration number:**NCT04433104.
26

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.

1 Introduction

2 Chronic obstructive pulmonary disease (COPD) is described – but not defined – as one of the major chronic
3 lung diseases characterized by persistent and progressive airflow obstruction. It is caused by an elevated chronic
4 pulmonary inflammatory response in the airways and bronchial structure to noxious particles or gases. The
5 pathological hallmarks of the disease include obstructive bronchiolitis, emphysema, and mucus hypersecretion¹.
6 Despite many medical advancements and technological improvements, our understanding of the pathological
7 mechanisms underlying the progressive and detrimental development of COPD remains incomplete, the definition of
8 the disease is controversial, diagnostic tests are inaccurate and unstandardized, and the treatment is inadequate². A
9 recent report stated that the global prevalence of COPD increased by 44% within the last 20 years, and more than 3.2
10 million patients died each year from COPD worldwide (accounting for approximately 5% of all deaths globally per
11 year)³. In Vietnam, according to the WHO report, 7.1% of males and 1.9% of females aged 40 and above are diagnosed
12 with COPD. Consequently, approximately 25% of hospital beds in respiratory wards are required for COPD patients,
13 resulting in a heavy burden to Vietnamese Medical Infrastructure and reducing patients' health and quality of life⁴.
14 The current pharmacological medications for COPD include the use of inhaled bronchodilator drugs, such as long-
15 acting β agonists (LABAs) and long-acting muscarinic antagonists (LAMAs), the use of inhaled corticosteroids (ICSs)
16 or a combination of these medications. Although it is generally accepted that pharmacological interventions via
17 inhalation would allow the accurate delivery of drugs and increase the clinical benefits, incorrect inhaler technique
18 and a lack of adherence when feeling healthy caused worse dyspnea, impaired health condition, and increased the
19 frequency of exacerbations and hospitalizations in Vietnamese COPD patients⁵. Therefore, identifying novel effective
20 therapies for COPD patients is urgent and important.

21 Since their first discovery in 1968, mesenchymal stem/stromal cells (MSCs) have been intensively studied
22 because of their therapeutic and regenerative features. The nomenclature of MSCs has been debated recently due to
23 not only the biological features of the MSCs themselves but also the medical abuse of the term “stem cells” inferring
24 direct medical benefit⁶. To standardize the characterization of MSCs and facilitate their therapeutic implications, the
25 International Society for Cellular Therapy (ISCT) has proposed the minimum criteria to define human MSCs^{7,8}. In
26 our study, MSCs were defined as mesenchymal stem/stromal cells, which are a class of adult mesenchymal progenitor
27 cells derived from either bone marrow, adipose, or umbilical cord tissue and met the minimum criteria of ISCT.
28 Among various sources of MSCs, human umbilical cord-derived MSCs (hUC-MSCs) are potentially more advanced
29 than their adult counterparts (bone marrow or adipose) for several reasons: (1) ease of collection as it is a noninvasive
30 process, (2) waiving ethical barriers as UC is medical waste discarded at birth, (3) rapid proliferation rate, (4)
31 maintenance of normal karyotype during prolonged culture *in vitro*, and (4) higher paracrine potency than adult tissue-
32 derived MSCs⁹. The therapeutic potential of hUC-MSCs has been proven in clinical studies, especially animal
33 pulmonary disease models, including acute respiratory distress syndrome (ARDS), bronchopulmonary dysplasia
34 (BPD), and COPD. It has been reported that UC-MSCs are effective in reducing lung inflammation and fibrosis
35 processes, preventing secondary infection, decreasing immune system damage, increasing bronchoalveolar fluid
36 clearance, and enhancing the regeneration of alveolar epithelium layers¹⁰⁻¹². The majority of intravenously

1 administered MSCs reportedly remain in the lung, especially pulmonary microvessels, which potentially contribute to
2 their beneficial effects in pulmonary disease models¹³. Hence, the safety and therapeutic effects of UC-MSC
3 administration for COPD require further investigation and clarification.

4 To date, five completed clinical trials have used bone marrow mononuclear cells (BMMCs), bone marrow-
5 derived MSCs (BM-MSCs), and UC-MSCs in COPD¹⁴⁻¹⁹. Although these clinical trials provided an enormous amount
6 of data supporting the safety of the therapy in the treatment of COPDs, the efficacy of the treatments remained
7 controversial due to several limitations, including trial design, lack of standardization of cell numbers administered to
8 patients, timing of MSC administration, and, most importantly, the lack of a control group in several studies. Moreover,
9 the variations in patient selection based on the severity and stage of COPD could be attributed to the effectiveness of
10 the cell therapy, resulting in caution in data interpretation. Last but not least, the quality of administered MSCs also
11 plays a significant role in the effectiveness of the treatment, i.e., the status of the cells (fresh culture vs. frozen cells),
12 cell sources (from young healthy donors or aging individuals), dosage frequency, etc. Therefore, identification of the
13 potential sources of MSCs (such as UC-MSCs), larger sample size with matched controls, and standardized
14 classification of COPD using international accepted criteria is required to further investigate the safety and efficacy
15 of MSC therapy. Based on preclinical studies and previous promising findings, we designed a matched control phase
16 I/II clinical trial to evaluate the safety and potential efficacy of the intravenous infusion of allogeneic hUC-MSCs in
17 patients with COPD characterized based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2019
18 criteria²⁰.

19 **Methods and analysis**

20 *Study objectives*

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

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

28 *Study design and ethics*

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

34 *Sample size*

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3 1 As a previous study indicated that the FEV₁ (%) of COPD patients was reduced to 35.4±7.1% (6% reduction)
4 2 after 6 months post-administration, we set this indicator at 18% reduction after 12 months post-administration to
5 3 calculate the minimum sample size for the proposed study^{18 21}. According to the continuous endpoint of two
6 4 independent sample studies²², we assumed α was 0.05 and type-II error β was 0.2; thus, the smallest sample size was
7 5 40 patients. The calculated sample size was 20 for each group.
8
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10 11 **Matching strategy:**

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13 7 The patients from the control group will be assigned to a patient from the MSC group once they meet all
14 8 matched criteria based on age (± 5 years), gender, and COPD severity classification (GOLD 2019). Patients from both
15 9 groups will receive standard COPD medication management according to their COPD severity classification and based
16 10 on the Vietnam Ministry of Health guideline for COPD treatment, as shown in Figure 2 and Table 1.
17

18
19 11 A total of 40 patients will be recruited and assigned to the UC-MSC administration group (20 patients) and the
20 12 control group (20 patients). The trial contains two phases: (1) the first phase will include recruiting and evaluating the
21 13 first 5 patients from each group to assess the safety of UC-MSC administration after 1 month of follow-up, and (2)
22 14 the second phase will be initiated after the 1st phase safety report is approved by the Ethical Committee of Vinmec
23 15 International Hospital and Vietnam Ministry of Health to start recruiting the remaining 15 patients from each group
24 16 to evaluate both safety and efficacy of the treatment.
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28 29 **Participants**

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31 18 The principal investigators, research and clinician team members are responsible for the study design, patient
32 19 screening, recruitment, conduct, and perform follow-up assessments in the trial. Participants will be allowed to enroll
33 20 or withdraw at any time throughout the study. The participants will have all screening and testing costs related to the
34 21 trials waived except for the costs of COPD medications or drugs. All participants' information will be protected by
35 22 coding and restricted access using a computer-based system. Participants will be enrolled in the study once they meet
36 23 all inclusion and exclusion criteria.
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40 41 **Inclusion and exclusion criteria**

42 25 The diagnostic criteria and severity classification of COPD refers to the criteria established by the COPD 2019
43 26 guidelines²⁰. Patients will be asked to confirm the COPD conditions and classification from national hospitals and
44 27 send the results to the administration office prior to enrollment in the trial for prescreening. Patients will be enrolled
45 28 in the study in compliance with the inclusion and exclusion criteria established by a screening protocol as presented
46 29 below.
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50 51 **Inclusion Criteria**

- 52 31
- 53 32 ▪ Diagnosed with COPD with stage B, C, or D according to GOLD 2019.
 - 54 33 ▪ Age between 40-75 years old.
 - 55 34 ▪ Both genders.
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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-MS, (3) number of participants, study duration, and classification into either MS administration or control group, (4) study procedure (including screening, COPD

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

9 ***Intervention***

10 30 Umbilical cord (UC) samples were obtained from healthy women with an uncomplicated, at term pregnancy
11 who underwent serological testing, including tests for HIV, cytomegalovirus (CMV), Epstein-Barr virus (EBV),
12 hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis, and chlamydia, at 38 weeks of
13 pregnancy, as shown in previous study²³. The UC tissues were collected at delivery and transferred to the Stem Cell
14 Core Facility at the Vinmec Research Institute of Stem Cell and Gene Technology under ISO 14644-1 (certification
15 number: CR61119-1). To generate a UC-MSC line for the current study, a single eligible UC tissue will be processed,
16 isolated and cultured under xeno-free and serum-free conditions as previously described²³. UC-MSCs will be
17 expanded under these conditions to passage 5 (P5) and cryopreserved in the serum- and xeno-free defined reagent
18 CryoStore[®] CS10 (Stem Cell Technology, Canada) in liquid nitrogen (gas phase) in an automated Brooks System
19 (Brooks Life Science, USA) for long-term storage. The releasing criteria for UC-MSC products are shown in Table
20 3.

21 To prepare UC-MSCs for therapy, aliquots of P5 UC-MSCs will be thawed in a temperature control water bath
22 or incubator on the infusion day. The hUC-MSCs will be washed and suspended in 0.9% normal saline. In addition to
23 inspecting the quality of the UC-MSC product based on the releasing criteria, the staff of the Cell Therapy Department
24 will confirm the viability and quality of the UC-MSC product before the infusion. The cell dose will be calculated
25 based on patients' body weight and cell viability results to obtain the dose of 1×10^6 viable cells/kg patient body weight
26 prior to transport to the administration ward. Currently, there is no effective treatment for COPD patients. Thus, the
27 intervention group will be given the standard COPD medication management as primary treatment and extra UC-
28 MSC administration, while the control group will receive only the standard COPD treatment (Table 1).

29 ***Mode of cell administration (UC-MSC group)***

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

34 ***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 SpO₂ 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

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3 1 analysis (including pH, PaO₂, PaCO₂, BE, HCO₃⁻), (4) respiratory functions (FEV₁, FEV₁/FVC, VC, TLC, RV,
4 2 DLCO, DLNO/DLCO), (5) electrocardiogram, echocardiography, high-resolution chest computed tomography,
5 3 abdominal ultrasound, abnormality of thyroid and mammary gland, (6) inflammatory response (CRP, Pro-BNP, and
6 4 Troponin-T) and (7) cytokine analysis from patients' plasma. In addition, the modified medical research council
7 5 (mMRC) questionnaire and quality of life (Georges Respiratory Questionnaire – SGRQ) will be used to monitor
8 6 respiratory function improvement. To reveal the therapeutic effects of UC-MSC administration, UC-MSC
9 7 characterization will be conducted *in vitro*, including MSC marker analysis, metabolic evaluation, immunoregulatory
10 8 assessment, and cytokine secretion analysis (Table 4).

9 ***Follow-up procedure***

10 Follow-up visits will be conducted at 3, 6, and 12 months after hUC-MSC administration. Patients will be
11 11 asked to come to the hospital to undergo an assessment of their conditions according to the protocol procedure. The
12 12 safety follow-up will include an extra 1-month follow-up point via telephone and outpatient contact, and patients will
13 13 only be asked to make an appointment if AEs or SAEs occur.

14 ***Data collection***

15 The data accumulated during the trial will be documented in the patients' medical records and the CRF. The
16 16 quality control officers from Vinmec Times City International Hospital and Vinmec Scientific Research Board
17 17 independently checked the accuracy and consistency of the CRF data with the original patients' medical records to
18 18 ensure that the data were accurately entered into the CRF. Once the CRF is checked, within 7 days, all data will be
19 19 recorded to RedCap software by assigned personnel and crosschecked by principle investigators. There are four data
20 20 collection points, including baseline and 3 months, 6 months, and 12 months postadministration. The internal auditor
21 21 of the Vinmec Research Institute of Stem Cell and Gene Technology will review each original research record to
22 22 confirm the accuracy, consistency, timely records, and meet the standard requirements. Data analysis will be
23 23 performed using RedCap and statistical analysis software following the statistical analysis strategy
24 24 (<https://redcap.vinmec.com/>). The data of this clinical trial will be disseminated with permission from funding bodies
25 25 and principle investigators through national and international conferences, peer-reviewed publications, and scientific
26 26 reports. A complete data set will be available upon request after trial completion.

27 ***Statistical analysis strategy***

28 Descriptive statistics will be used to illustrate the demographics of the COPD patients. Categorical variables
29 29 are expressed as proportions, whereas quantitative variables are described as the mean values and their standard
30 30 deviations or as the medians and their interquartile ranges. The number and type of adverse events/serious adverse
31 31 events will be compared between the two treatment groups using the Chi square (or Fisher's exact) test. For the
32 32 intervention and control groups, the indicators (m-MRC, CAT, SGRQ, respiratory functions, and arterial blood gas
33 33 analysis) at baseline and at 3 months, 6 months, and 12 months will be compared with repeated measures ANOVA.
34 34 P-values < 0.05 will be considered statistically significant. The analyses will be performed using Stata version 14
35 35 (StataCorp, College Station, TX, USA).

1 **Patient and public involvement**

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

4 **Ethics and Dissemination**

5 This trial, including the consent form and clinical protocol, was approved by the Ethical Committee of Vinmec
6 International Hospital (number: 166/2019/QĐ-VMEC) and Vietnam Ministry of Health (number: 2002/QĐ-BYT).
7 This study was registered at ClinicalTrials.gov (number NCT04433104). The trial conforms with the Declaration of
8 Helsinki. All participants will provide oral and written informed consent prior to participating in the study. This study
9 will be reported in accordance with the STROBE guidelines for matched case-control trial²⁴. We will disseminate the
10 research results through high-quality peer-reviewed open access (via PubMed) journals and presentations at national
11 and international conferences. Finally, an ongoing update of the trial will also be provided and shared annually with
12 our partners in the health system and community agencies according to National Regulation.

13 **Discussion**

14 This study protocol presents the matched-control phase I/II clinical trial evaluating the safety and potential
15 efficacy of allogeneic UC-MSC administration in patients with moderate-to-severe COPD (GOLD 2019). To date,
16 there is no effective treatment available for COPD patients, and pharmacological interventions are hampered by the
17 heterogeneity of disease mechanisms and phenotypic alternation. Therefore, establishing new treatment methods to
18 reduce the devastating effects of COPD is imperative. The body of preclinical studies and human clinical trials
19 suggests that MSC administration emerges as a potential therapeutic approach for COPD because MSCs have been
20 found to be well tolerated and safe in many clinical trials and have proven their effectiveness in animal models. Several
21 clinical trials have been conducted in COPD. Most of these studies were phase 1 safety trials, which uniformly reported
22 no obvious adverse events and serious adverse events as well as no evidence of infusional toxicities during the follow-
23 up period²⁵. However, the effectiveness of MSC therapy showed differences among various clinical trials, and a small
24 number of trials have revealed no significant changes in lung function and fibrosis postadministration compared with
25 baseline levels²⁶. Therefore, it is important to comprehensively analyze the factors that directly contribute to treatment
26 safety and efficacy.

27 Human clinical trials were conducted to evaluate the safety and efficacy of MSCs in the treatment of COPD,
28 including five studies using bone marrow-derived cells¹⁴⁻¹⁷ and a pilot study using UC-MSCs¹⁸. The first study
29 supported the safety profile of MSCs administered BMSCs to four COPD patients, although the overall clinical
30 outcomes did not demonstrate the efficacy of the treatment. It is understandable that studies together with the two
31 trials (NCT001110252 and NCT01306513) are phase 1 clinical trials that aimed to evaluate the safety and feasibility
32 of cellular administration in the treatment of COPD. Notably, the NCT001110252 study followed up with patients for
33 up to 3 years illustrated an overall reduction in the process of COPD pathological development¹⁹. In a pilot study
34 using UC-MSCs, COPD patients were followed up for 6 months, and no AEs or SAEs were observed throughout the
35 course of the study. Although clinical outcomes such as COPD exacerbations, mMRC score, and CAT were

1 significantly reduced postadministration, pulmonary function parameters remained unchanged compared to baseline
2
3 1⁸. In our current study, we use UC-MSCs as an “off-the-shelf” product for administration, providing flexibility in
4 2
5 3 patient management and standardized products for all treated patients, allowing more accuracy in evaluation.
6 4
7 Moreover, by using a matched control design, our study aims to eliminate the variability in COPD conditions between
8 5
9 the intervention and control groups to accurately evaluate the safety and efficacy of the treatment. In general, it was
10 6
11 confirmed that MSC administration is well tolerated without serious adverse events or administration-associated
12 7
13 adverse events and is not associated with significant alterations in spirometry, immune function, cardiovascular
14 8
15 activity, or patient quality of life ²⁷.

16 9 The major delivery routes of MSCs in the treatment of pulmonary disease include intraperitoneal (usually in
17 10
18 animal models), intranasal or intratracheal, and IV administration. The intratracheal administration of MSCs was
19 11
20 performed in children with bronchopulmonary dysplasia in several small uncontrolled studies. However, in terms of
21 12
22 COPD, all trials utilized IV administration with the aim of investigating whether systemic administration of MSCs is
23 13
24 safe and effective in COPD patients. In fact, the IV administration route is considered a better option compared to
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26 intratracheal delivery for several reasons. Previous studies illustrated that IV administration of MSCs was safe and
27 15
28 potentially provided therapeutic effects in several lung diseases, including COPD ^{15,26}. Moreover, a systemic analysis
29 16
30 of preclinical studies suggested that IV administration of MSCs introduced better effects than those administered via
31 17
32 the intratracheal route ¹³. We hypothesized that the results of this clinical trial will provide data supporting that UC-
33 18
34 MSC administration via the IV route is safe, feasible, and potentially effective in COPD patients.

35 19 The dose-escalating evaluation has been conducted in several clinical trials for various diseases, including
36 20
37 pulmonary syndromes, using a wide range of UC-MSC doses from 0.5 – 10 million cells/kg via IV administration ¹⁵
38 21
39 ^{26,28}. Notably, limited studies have reported the different effects of MSC doses in COPD patients. In fact, a relatively
40 22
41 high dose (10 million cells/kg patient body weight) was tested in ARDS patients without any administration-associated
42 23
43 AEs or SAEs recorded. However, it is important to note that delivery of a high dose of stem cells might increase the
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45 risk of pulmonary embolism and thrombosis regardless of administration route, which was demonstrated previously
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47 in animal models and clinical trials ²⁹⁻³¹. Therefore, in this trial, we used the most common dose of MSCs used in
48 26
49 numerous studies, which is 1 million cells/kg patient body weight.

50 27 In pre-clinical models of elastase-induced emphysema, two doses of MSCs improved anti-inflammatory
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52 regulation and lung recover compared to a single dose of MSCs ³². In clinical trial using BM-MSCs, COPD patients
53 29
54 received four infusions showed a reduction in circulating C-reactive protein and in combination with lung volume
55 30
56 reduction strategy, two infusions were enough to increase the expression of CD31, an indication of microvascular
57 31
58 endothelial cell response ^{17,25}. Moreover, the effects of autologous MSC administration were reported to be relatively
59 32
60 narrow because it was reported the positive effects in patients with type 2 diabetes were observed as early as 1 month
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62 post-administration of MSCs and started to diminish after three to six months post-administration ^{33,34}. Hence, in this
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64 current study, we will perform two doses of UC-MSCs with a 3-month intervening interval.

65 35 This clinical trial has several advantages. First, this is the first trial using an “off-the-shelf” product (UC-MSCs)
66 36
67 for COPD patients. Second, this is the first trial to investigate the therapeutic effects of UC-MSCs as supplementary

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

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11 control and UC-MSC experiment set up.

12 **Contributors:**

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

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20 **Conflicts of Interests**

21 None declared.

22 **Patient consent for publication**

23 Not required

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19 9 **Figure Legend:**

20 10 **Figure 1: Schematic of the study.** COPD patients will be screened to enroll in the study. Patients from the control
21 11 group will be assigned to a patient from the UC-MSC group once they meet all matched criteria based on age (± 5
22 12 years), gender, and COPD severity classification (GOLD 2019).

23 13 **Figure 2: Standard COPD medication treatment for both groups according to GOLD 2019 and Vietnam**
24 14 **Ministry of Health Guideline.** Matched COPD patients will be treated using the same treatment based on their GOLD
25 15 2019 classification (Groups A, B, C, and D). Group A (not included in this study): a single bronchodilator will be used
26 16 and based on the clinical assessments and persistence of the symptoms to continue/stop or replace by another
27 17 bronchodilator. Group B: Single LAMA or LABA will be initially used. If the symptoms are not reduced, a
28 18 combination of both LAMA and LABA will be applied. Group C: A single LAMA drug will be used for initial
29 19 treatment. If exacerbations occur, LAMA and LABA combination will be applied as priority. The LAMA + ICS will
30 20 be applied in specific cases based on clinical assessment, as the ICS has been reported to have severe side effects on
31 21 lung inflammation. Group D: Should start the treatment with LAMA. If the patient has CAT >20 , LABA and LAMA
32 22 will be used as initial treatment. LABA + ICS will be used as the initial treatment only when the patient has asthma
33 23 COPD overlap or the patient's eosinophil level > 300 . If exacerbation occurs after the initial treatment, the combination
34 24 of LAMA, LABA, and ICS should be applied. Additional roflumilast should be used if FEV1 $< 50\%$ and the patient
35 25 has chronic bronchitis. Macrolide should be used if the patient is a former smoker. The red arrow indicates priority
36 26 treatment.
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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: <ul style="list-style-type: none"> ▪ 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			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		
Medication treatment ²			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Informed consent		<input checked="" type="checkbox"/>				
Inclusion and exclusion criteria		<input checked="" type="checkbox"/>				
Demographic information		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			
Patients' medical reports		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Vital signs ³ /physical examination		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
COPD assessment	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>				
COPD GOLD 2019 classification	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Hematology analysis ⁴	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Infectious disease examination/test ⁵	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			
Blood oxygen saturation/arterial blood gas analysis ⁶	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Chest CT scan		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Chest X-ray		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Pulmonary function analysis		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Adverse event evaluation			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Mortality/complications monitoring			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

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.

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

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

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9: Infectious diseases include hepatitis, syphilis, HIV, HBV, and tuberculosis.

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11: Blood gas analysis includes pH, PaO₂, PaCO₂, BE, HCO₃⁻.

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For peer review only

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)		
<i>CD73</i>		> 95%
<i>CD90</i>	Flow cytometry using the Human MSC	> 95%
<i>CD105</i>	Analysis Kit (Becton Dickinson, USA)	> 95%
Negative markers (%)		< 2%
Cell viability (%) (mean \pm SD)	Trypan Blue staining	> 80%
Microorganism tests	BacT/Alert® 3D microbial detection System (Biomerieux, USA)	Negative
Mycoplasma	MycoAlert™ Plus Mycoplasma Detection Kit (Lonza, Switzerland)	Negative
Endotoxin	Endosafe-PTS (Charles River Laboratories)	\leq 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/ <i>In vitro</i> 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.

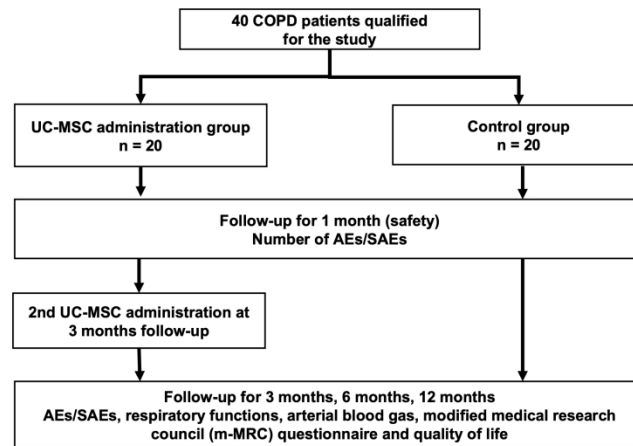


Figure 1

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).

190x275mm (300 x 300 DPI)

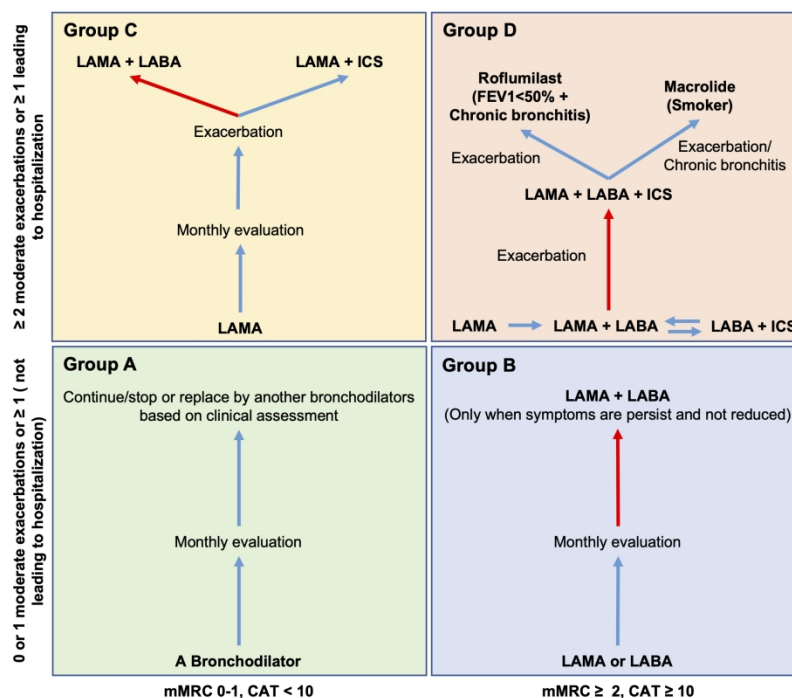


Figure 2:

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 GOLD 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

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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.

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		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

1	Roles and	#5b	Name and contact information for the trial sponsor	14
2	responsibilities:			
3	sponsor contact			
4	information			
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7				
8	Roles and	#5c	Role of study sponsor and funders, if any, in study design;	14
9	responsibilities:		collection, management, analysis, and interpretation of data;	
10	sponsor and funder		writing of the report; and the decision to submit the report for	
11			publication, including whether they will have ultimate authority	
12			over any of these activities	
13				
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15				
16	Roles and	#5d	Composition, roles, and responsibilities of the coordinating	14
17	responsibilities:		centre, steering committee, endpoint adjudication committee,	
18	committees		data management team, and other individuals or groups	
19			overseeing the trial, if applicable (see Item 21a for data	
20			monitoring committee)	
21				
22				
23				
24	Introduction			
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26				
27	Background and	#6a	Description of research question and justification for undertaking	4
28	rationale		the trial, including summary of relevant studies (published and	
29			unpublished) examining benefits and harms for each intervention	
30				
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32	Background and	#6b	Explanation for choice of comparators	4-5
33	rationale: choice of			
34	comparators			
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37	Objectives	#7	Specific objectives or hypotheses	5
38				
39				
40	Trial design	#8	Description of trial design including type of trial (eg, parallel	5, 6
41			group, crossover, factorial, single group), allocation ratio, and	
42			framework (eg, superiority, equivalence, non-inferiority,	
43			exploratory)	
44				
45				
46	Methods:			
47	Participants,			
48	interventions, and			
49	outcomes			
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53	Study setting	#9	Description of study settings (eg, community clinic, academic	5, 6
54			hospital) and list of countries where data will be collected.	
55			Reference to where list of study sites can be obtained	
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1	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
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6	Interventions:	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
7	description			
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10	Interventions:	#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
11	modifications			
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15	Interventions:	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	8, 9
16	adherence			
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20	Interventions:	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8, 9
21	concomitant care			
22				
23				
24	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
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34	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
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40	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
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45	Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7,8
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49	Methods: Assignment			
50	of interventions (for			
51	controlled trials)			
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53				
54	Allocation: sequence	#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"
55	generation			
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provided in a separate document that is unavailable to those who enrol participants or assign interventions

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4	Allocation	#16b	Mechanism of implementing the allocation sequence (eg, central
5	concealment		telephone; sequentially numbered, opaque, sealed envelopes),
6			describing any steps to conceal the sequence until interventions
7	mechanism		are assigned
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11	Allocation:	#16c	Who will generate the allocation sequence, who will enrol
12	implementation		participants, and who will assign participants to interventions
13			
14	Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial
15			participants, care providers, outcome assessors, data analysts),
16			and how
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20	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is permissible,
21	emergency unblinding		and procedure for revealing a participant's allocated intervention
22			during the trial
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25	Methods: Data		
26	collection,		
27	management, and		
28	analysis		
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32	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and
33			other trial data, including any related processes to promote data
34			quality (eg, duplicate measurements, training of assessors) and a
35			description of study instruments (eg, questionnaires, laboratory
36			tests) along with their reliability and validity, if known.
37			Reference to where data collection forms can be found, if not in
38			the protocol
39			
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42			
43	Data collection plan:	#18b	Plans to promote participant retention and complete follow-up,
44	retention		including list of any outcome data to be collected for participants
45			who discontinue or deviate from intervention protocols
46			
47			
48	Data management	#19	Plans for data entry, coding, security, and storage, including any
49			related processes to promote data quality (eg, double data entry;
50			range checks for data values). Reference to where details of data
51			management procedures can be found, if not in the protocol
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55	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary
56			outcomes. Reference to where other details of the statistical
57			analysis plan can be found, if not in the protocol
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1	Statistics: additional	#20b	Methods for any additional analyses (eg, subgroup and adjusted	10
2	analyses		analyses)	
3				
4	Statistics: analysis	#20c	Definition of analysis population relating to protocol non-	10
5	population and missing		adherence (eg, as randomised analysis), and any statistical	
6	data		methods to handle missing data (eg, multiple imputation)	
7				
8				
9				
10	Methods: Monitoring			
11				
12	Data monitoring:	#21a	Composition of data monitoring committee (DMC); summary of	10
13	formal committee		its role and reporting structure; statement of whether it is	
14			independent from the sponsor and competing interests; and	
15			reference to where further details about its charter can be found,	
16			if not in the protocol. Alternatively, an explanation of why a	
17			DMC is not needed	
18				
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22	Data monitoring:	#21b	Description of any interim analyses and stopping guidelines,	10
23	interim analysis		including who will have access to these interim results and make	
24			the final decision to terminate the trial	
25				
26				
27	Harms	#22	Plans for collecting, assessing, reporting, and managing solicited	9
28			and spontaneously reported adverse events and other unintended	
29			effects of trial interventions or trial conduct	
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33	Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and	9, 10
34			whether the process will be independent from investigators and	
35			the sponsor	
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38	Ethics and			
39	dissemination			
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42	Research ethics	#24	Plans for seeking research ethics committee / institutional review	10
43	approval		board (REC / IRB) approval	
44				
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46	Protocol amendments	#25	Plans for communicating important protocol modifications (eg,	10, 11
47			changes to eligibility criteria, outcomes, analyses) to relevant	
48			parties (eg, investigators, REC / IRBs, trial participants, trial	
49			registries, journals, regulators)	
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53	Consent or assent	#26a	Who will obtain informed consent or assent from potential trial	5, 6
54			participants or authorised surrogates, and how (see Item 32)	
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1	Consent or assent:	#26b	Additional consent provisions for collection and use of	5,6
2	ancillary studies		participant data and biological specimens in ancillary studies, if	
3			applicable	
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6	Confidentiality	#27	How personal information about potential and enrolled	10
7			participants will be collected, shared, and maintained in order to	
8			protect confidentiality before, during, and after the trial	
9				
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11	Declaration of interests	#28	Financial and other competing interests for principal investigators	14
12			for the overall trial and each study site	
13				
14				
15	Data access	#29	Statement of who will have access to the final trial dataset, and	10
16			disclosure of contractual agreements that limit such access for	
17			investigators	
18				
19				
20	Ancillary and post trial	#30	Provisions, if any, for ancillary and post-trial care, and for	"n/a"
21	care		compensation to those who suffer harm from trial participation	
22				
23				
24	Dissemination policy:	#31a	Plans for investigators and sponsor to communicate trial results	10
25	trial results		to participants, healthcare professionals, the public, and other	
26			relevant groups (eg, via publication, reporting in results	
27			databases, or other data sharing arrangements), including any	
28			publication restrictions	
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33	Dissemination policy:	#31b	Authorship eligibility guidelines and any intended use of	14
34	authorship		professional writers	
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37	Dissemination policy:	#31c	Plans, if any, for granting public access to the full protocol,	"n/a"
38	reproducible research		participant-level dataset, and statistical code	
39				
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41	Appendices			
42				
43	Informed consent	#32	Model consent form and other related documentation given to	"n/a"
44	materials		participants and authorised surrogates	
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47	Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of	Table 2
48			biological specimens for genetic or molecular analysis in the	
49			current trial and for future use in ancillary studies, if applicable	
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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:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2020-045788.R2
Article Type:	Protocol
Date Submitted by the Author:	16-Apr-2021
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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3 **Allogeneic human umbilical cord-derived mesenchymal stem/stromal cells for chronic obstructive pulmonary**
4 **disease (COPD): study protocol for a matched case-control, phase I/II trial.**
5

6 Duc M. Hoang^{1*}, Kien T. Nguyen^{1*}, Anh H. Nguyen², Bach N. Nguyen², Liem Nguyen Thanh^{*1}.
7

8 *These authors contributed equally to the work.
9

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11

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17

18
19 **Keywords:** Umbilical cord-derived Mesenchymal Stem/stromal Cells, Chronic Obstructive Pulmonary Disease
20 (COPD), allogeneic MSC administration, clinical trial.
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23 **Word count:** 5599 words
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3 **1 Abstract**

4
5 **2 Introduction**

6
7 3 The global prevalence of chronic obstructive pulmonary disease (COPD) is increasing, and it has become a
8 4 major public health burden worldwide, including in Vietnam. A large body of preclinical and clinical studies
9 5 supports the safety of mesenchymal stem/stromal cells (MSCs) in the treatment of lung injury, including COPD. The
10 6 aim of this trial is to investigate the safety and potential therapeutic efficacy of allogeneic administration of
11 7 umbilical cord-derived MSCs (UC-MSCs) as a supplementary intervention in combination with standard COPD
12 8 medication treatments in patients with moderate-to-severe COPD based on the Global Initiative for Chronic
13 9 Obstructive Lung Disease (GOLD) 2019 and Vietnam Ministry of Health's guidelines.

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18 **10 Methods and analysis**

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20 11 This matched case-control phase I/II trial is conducted at Vinmec Times City International Hospital, Hanoi,
21 12 Vietnam between June 2020 and December 2021. In this study, 40 patients will be enrolled and assigned into two
22 13 age-, gender- and COPD condition-matched groups, including a UC-MSC group and a control group. Both groups
23 14 will receive standard COPD medication treatment based on the GOLD 2019 guidelines and the Vietnam Ministry of
24 15 Health protocol. The UC-MSC group will receive two doses of thawed UC-MSC product with an intervention
25 16 interval of 3 months. The primary outcome measures will include the incidence of prespecified administration-
26 17 associated adverse events (AEs) and serious adverse events (SAEs). The efficacy will be evaluated based on the
27 18 absolute changes in the number of admissions, arterial blood gas analysis, lung function and lung fibrosis via CT
28 19 scan and chest X-ray. The clinical evaluation will be conducted at baseline and 3, 6, and 12 months post
29 20 intervention.

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35 **21 Ethics and dissemination**

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37 22 Ethical approval was secured from the Ethical Committee of Vinmec International Hospital
38 23 (number:166/2019/QĐ-VMC) and Vietnam Ministry of Health (number:2002/QĐ-BYT). The results will be
39 24 reported to trial collaborators, publication in peer-reviewed academic journals.

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42 **25 Trial registration number:**NCT04433104.
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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.

1 Introduction

2 Chronic obstructive pulmonary disease (COPD) is described – but not defined – as one of the major chronic
3 lung diseases characterized by persistent and progressive airflow obstruction. It is caused by an elevated chronic
4 pulmonary inflammatory response in the airways and bronchial structure to noxious particles or gases. The
5 pathological hallmarks of the disease include obstructive bronchiolitis, emphysema, and mucus hypersecretion¹.
6 Despite many medical advancements and technological improvements, our understanding of the pathological
7 mechanisms underlying the progressive and detrimental development of COPD remains incomplete, the definition of
8 the disease is controversial, diagnostic tests are inaccurate and unstandardized, and the treatment is inadequate². A
9 recent report stated that the global prevalence of COPD increased by 44% within the last 20 years, and more than 3.2
10 million patients died each year from COPD worldwide (accounting for approximately 5% of all deaths globally per
11 year)³. In Vietnam, according to the WHO report, 7.1% of males and 1.9% of females aged 40 and above are diagnosed
12 with COPD. Consequently, approximately 25% of hospital beds in respiratory wards are required for COPD patients,
13 resulting in a heavy burden to Vietnamese Medical Infrastructure and reducing patients' health and quality of life⁴.
14 The current pharmacological medications for COPD include the use of inhaled bronchodilator drugs, such as long-
15 acting β agonists (LABAs) and long-acting muscarinic antagonists (LAMAs), the use of inhaled corticosteroids (ICSs)
16 or a combination of these medications. Although it is generally accepted that pharmacological interventions via
17 inhalation would allow the accurate delivery of drugs and increase the clinical benefits, incorrect inhaler technique
18 and a lack of adherence when feeling healthy caused worse dyspnea, impaired health condition, and increased the
19 frequency of exacerbations and hospitalizations in Vietnamese COPD patients⁵. Therefore, identifying novel effective
20 therapies for COPD patients is urgent and important.

21 Since their first discovery in 1968, mesenchymal stem/stromal cells (MSCs) have been intensively studied
22 because of their therapeutic and regenerative features. The nomenclature of MSCs has been debated recently due to
23 not only the biological features of the MSCs themselves but also the medical abuse of the term “stem cells” inferring
24 direct medical benefit⁶. To standardize the characterization of MSCs and facilitate their therapeutic implications, the
25 International Society for Cellular Therapy (ISCT) has proposed the minimum criteria to define human MSCs^{7,8}. In
26 our study, MSCs were defined as mesenchymal stem/stromal cells, which are a class of adult mesenchymal progenitor
27 cells derived from either bone marrow, adipose, or umbilical cord tissue and met the minimum criteria of ISCT.
28 Among various sources of MSCs, human umbilical cord-derived MSCs (hUC-MSCs) are potentially more advanced
29 than their adult counterparts (bone marrow or adipose) for several reasons: (1) ease of collection as it is a noninvasive
30 process, (2) waiving ethical barriers as UC is medical waste discarded at birth, (3) rapid proliferation rate, (4)
31 maintenance of normal karyotype during prolonged culture *in vitro*, and (4) higher paracrine potency than adult tissue-
32 derived MSCs⁹. The therapeutic potential of hUC-MSCs has been proven in clinical studies, especially animal
33 pulmonary disease models, including acute respiratory distress syndrome (ARDS), bronchopulmonary dysplasia
34 (BPD), and COPD. It has been reported that UC-MSCs are effective in reducing lung inflammation and fibrosis
35 processes, preventing secondary infection, decreasing immune system damage, increasing bronchoalveolar fluid
36 clearance, and enhancing the regeneration of alveolar epithelium layers¹⁰⁻¹². The majority of intravenously

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3 1 administered MSCs reportedly remain in the lung, especially pulmonary microvessels, which potentially contribute to
4 2 their beneficial effects in pulmonary disease models¹³. Hence, the safety and therapeutic effects of UC-MSC
5 3 administration for COPD require further investigation and clarification.

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8 4 To date, five completed clinical trials have used bone marrow mononuclear cells (BMMCs), bone marrow-
9 5 derived MSCs (BM-MSCs), and UC-MSCs in COPD¹⁴⁻¹⁹. Although these clinical trials provided an enormous amount
10 6 of data supporting the safety of the therapy in the treatment of COPDs, the efficacy of the treatments remained
11 7 controversial due to several limitations, including trial design, lack of standardization of cell numbers administered to
12 8 patients, timing of MSC administration, and, most importantly, the lack of a control group in several studies. Moreover,
13 9 the variations in patient selection based on the severity and stage of COPD could be attributed to the effectiveness of
14 10 the cell therapy, resulting in caution in data interpretation. Last but not least, the quality of administered MSCs also
15 11 plays a significant role in the effectiveness of the treatment, i.e., the status of the cells (fresh culture vs. frozen cells),
16 12 cell sources (from young healthy donors or aging individuals), dosage frequency, etc. Therefore, identification of the
17 13 potential sources of MSCs (such as UC-MSCs), larger sample size with matched controls, and standardized
18 14 classification of COPD using international accepted criteria is required to further investigate the safety and efficacy
19 15 of MSC therapy. Based on preclinical studies and previous promising findings, we designed a matched control phase
20 16 I/II clinical trial to evaluate the safety and potential efficacy of the intravenous infusion of allogeneic hUC-MSCs in
21 17 patients with COPD characterized based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2019
22 18 criteria²⁰.

23 19 **Methods and analysis**

24 20 ***Study objectives***

25 21 The aim of this trial is to evaluate the safety and potential efficacy of allogeneic UC-MSC administration in patients
26 22 with COPD. There are three specific objectives:

- 27 23 1. Evaluate the safety and potential therapeutic effectiveness of intravenously (IV) administered UC-MSCs in
28 24 patients with COPD.
- 29 25 2. To prove the hypothesis that IV administration of UC-MSCs can improve lung function and reduce
30 26 inflammatory responses in the lungs and fibrosis.
- 31 27 3. Explore the potential therapeutic mechanism of UC-MSCs in the treatment of COPD.

32 28 ***Study design and ethics***

33 29 This matched case-control phase I/II clinical trial was approved by the Ethical Committee of Vinmec
34 30 International Hospital (number: 166/2019/QĐ-VMC) and Vietnam Ministry of Health (number: 2002/QĐ-BYT).
35 31 This study was registered at ClinicalTrials.gov (number NCT04433104). To achieve the aims, a total of 40 patients
36 32 with COPD will be recruited at the Internal Medicine Department at Vinmec Times City International Hospital, Hanoi,
37 33 Vietnam, between June 2020 and December 2021. A flowchart of the study design is shown in Figure 1.

38 34 ***Sample size***

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3 1 As a previous study indicated that the FEV₁ (%) of COPD patients was reduced to 35.4±7.1% (6% reduction)
4 2 after 6 months post-administration, we set this indicator at 18% reduction after 12 months post-administration to
5 3 calculate the minimum sample size for the proposed study^{18 21}. According to the continuous endpoint of two
6 4 independent sample studies²², we assumed α was 0.05 and type-II error β was 0.2; thus, the smallest sample size was
7 5 40 patients. The calculated sample size was 20 for each group.
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10 11 ***Matching strategy:*** 12

13 7 The patients from the control group will be assigned to a patient from the MSC group once they meet all
14 8 matched criteria based on age (± 5 years), gender, and COPD severity classification (GOLD 2019). Patients from both
15 9 groups will receive standard COPD medication management according to their COPD severity classification and based
16 10 on the Vietnam Ministry of Health guideline for COPD treatment, as shown in Figure 2 and Table 1.
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19 11 A total of 40 patients will be recruited and assigned to the UC-MSC administration group (20 patients) and the
20 12 control group (20 patients). The trial contains two phases: (1) the first phase will include recruiting and evaluating the
21 13 first 5 patients from each group to assess the safety of UC-MSC administration after 1 month of follow-up, and (2)
22 14 the second phase will be initiated after the 1st phase safety report is approved by the Ethical Committee of Vinmec
23 15 International Hospital and Vietnam Ministry of Health to start recruiting the remaining 15 patients from each group
24 16 to evaluate both safety and efficacy of the treatment.
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29 ***Participants*** 30

31 18 The principal investigators, research and clinician team members are responsible for the study design, patient
32 19 screening, recruitment, conduct, and perform follow-up assessments in the trial. Participants will be allowed to enroll
33 20 or withdraw at any time throughout the study. The participants will have all screening and testing costs related to the
34 21 trials waived except for the costs of COPD medications or drugs. All participants' information will be protected by
35 22 coding and restricted access using a computer-based system. Participants will be enrolled in the study once they meet
36 23 all inclusion and exclusion criteria.
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40 ***Inclusion and exclusion criteria*** 41

42 25 The diagnostic criteria and severity classification of COPD refers to the criteria established by the COPD 2019
43 26 guidelines²⁰. Patients will be asked to confirm the COPD conditions and classification from national hospitals and
44 27 send the results to the administration office prior to enrollment in the trial for prescreening. Patients will be enrolled
45 28 in the study in compliance with the inclusion and exclusion criteria established by a screening protocol as presented
46 29 below.
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50 ***Inclusion Criteria*** 51

- 52 31 ▪ Diagnosed with COPD with stage B, C, or D according to GOLD 2019.
- 53 32 ▪ Age between 40-75 years old.
- 54 33 ▪ Both genders.
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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

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

9 ***Intervention***

10 30 Umbilical cord (UC) samples were obtained from healthy women with an uncomplicated, at term pregnancy
11 who underwent serological testing, including tests for HIV, cytomegalovirus (CMV), Epstein-Barr virus (EBV),
12 hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis, and chlamydia, at 38 weeks of
13 pregnancy, as shown in previous study²³. The UC tissues were collected at delivery and transferred to the Stem Cell
14 Core Facility at the Vinmec Research Institute of Stem Cell and Gene Technology under ISO 14644-1 (certification
15 number: CR61119-1). To generate a UC-MSC line for the current study, a single eligible UC tissue will be processed,
16 isolated and cultured under xeno-free and serum-free conditions as previously described²³. UC-MSCs will be
17 expanded under these conditions to passage 5 (P5) and cryopreserved in the serum- and xeno-free defined reagent
18 CryoStore[®] CS10 (Stem Cell Technology, Canada) in liquid nitrogen (gas phase) in an automated Brooks System
19 (Brooks Life Science, USA) for long-term storage. The releasing criteria for UC-MSC products are shown in Table
20 3.

21 To prepare UC-MSCs for therapy, aliquots of P5 UC-MSCs will be thawed in a temperature control water bath
22 or incubator on the infusion day. The hUC-MSCs will be washed and suspended in 0.9% normal saline. In addition to
23 inspecting the quality of the UC-MSC product based on the releasing criteria, the staff of the Cell Therapy Department
24 will confirm the viability and quality of the UC-MSC product before the infusion. The cell dose will be calculated
25 based on patients' body weight and cell viability results to obtain the dose of 1×10^6 viable cells/kg patient body weight
26 prior to transport to the administration ward. Currently, there is no effective treatment for COPD patients. Thus, the
27 intervention group will be given the standard COPD medication management as primary treatment and extra UC-
28 MSC administration, while the control group will receive only the standard COPD treatment (Table 1).

29 ***Mode of cell administration (UC-MSC group)***

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

34 ***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 SpO₂ 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 reported²⁴. 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)

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3 1 The efficacy endpoints are as follows: (1) number of admissions and readmissions, (2) general self-efficacy,
4 2 (3) the number of admissions and unscheduled outpatient visits due to symptoms of COPD, (3) arterial blood gas
5 3 analysis (including pH, PaO₂, PaCO₂, BE, HCO₃⁻), (4) respiratory functions (FEV₁, FEV₁/FVC, VC, TLC, RV,
6 4 DLCO, DLNO/DLCO), (5) electrocardiogram, echocardiography, high-resolution chest computed tomography,
7 5 abdominal ultrasound, abnormality of thyroid and mammary gland, (6) inflammatory response (CRP, Pro-BNP, and
8 6 Troponin-T) and (7) cytokine analysis from patients' plasma. In addition, the modified medical research council
9 7 (mMRC) questionnaire and quality of life (Georges Respiratory Questionnaire – SGRQ) will be used to monitor
10 8 respiratory function improvement. To reveal the therapeutic effects of UC-MSC administration, UC-MSC
11 9 characterization will be conducted *in vitro*, including MSC marker analysis, metabolic evaluation, immunoregulatory
12 10 assessment, and cytokine secretion analysis (Table 4).

11 ***Follow-up procedure***

12 12 Follow-up visits will be conducted at 3, 6, and 12 months after hUC-MSC administration. Patients will be
13 13 asked to come to the hospital to undergo an assessment of their conditions according to the protocol procedure. The
14 14 safety follow-up will include an extra 1-month follow-up point via telephone and outpatient contact, and patients will
15 15 only be asked to make an appointment if AEs or SAEs occur.

16 ***Data collection***

17 17 The data accumulated during the trial will be documented in the patients' medical records and the CRF. The
18 18 quality control officers from Vinmec Times City International Hospital and Vinmec Scientific Research Board
19 19 independently checked the accuracy and consistency of the CRF data with the original patients' medical records to
20 20 ensure that the data were accurately entered into the CRF. Once the CRF is checked, within 7 days, all data will be
21 21 recorded to RedCap software by assigned personnel and crosschecked by principle investigators. There are four data
22 22 collection points, including baseline and 3 months, 6 months, and 12 months postadministration. The internal auditor
23 23 of the Vinmec Research Institute of Stem Cell and Gene Technology will review each original research record to
24 24 confirm the accuracy, consistency, timely records, and meet the standard requirements. Data analysis will be
25 25 performed using RedCap and statistical analysis software following the statistical analysis strategy
26 26 (<https://redcap.vinmec.com/>). The data of this clinical trial will be disseminated with permission from funding bodies
27 27 and principle investigators through national and international conferences, peer-reviewed publications, and scientific
28 28 reports. A complete data set will be available upon request after trial completion.

29 ***Statistical analysis strategy***

30 30 Descriptive statistics will be used to illustrate the demographics of the COPD patients. Categorical variables
31 31 are expressed as proportions, whereas quantitative variables are described as the mean values and their standard
32 32 deviations or as the medians and their interquartile ranges. The number and type of adverse events/serious adverse
33 33 events will be compared between the two treatment groups using the Chi square (or Fisher's exact) test. For the
34 34 intervention and control groups, the indicators (m-MRC, CAT, SGRQ, respiratory functions, and arterial blood gas
35 35 analysis) at baseline and at 3 months, 6 months, and 12 months will be compared with repeated measures ANOVA.

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3 1 P-values < 0.05 will be considered statistically significant. The analyses will be performed using Stata version 14
4 (StataCorp, College Station, TX, USA).
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6 ***Patient and public involvement***

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9 4 The patients and public were not involved in the design, or conduct, or reporting or dissemination plans of
10 our research.
11

12 **Ethics and Dissemination**

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14 7 This trial, including the consent form and clinical protocol, was approved by the Ethical Committee of Vinmec
15 International Hospital (number: 166/2019/QĐ-VMEC) and Vietnam Ministry of Health (number: 2002/QĐ-BYT).
16 8 This study was registered at ClinicalTrials.gov (number NCT04433104). The trial conforms with the Declaration of
17 9 Helsinki. All participants will provide oral and written informed consent prior to participating in the study. This study
18 10 will be reported in accordance with the STROBE guidelines for matched case-control trial²⁵. We will disseminate the
19 11 research results through high-quality peer-reviewed open access (via PubMed) journals and presentations at national
20 12 and international conferences. Finally, an ongoing update of the trial will also be provided and shared annually with
21 13 our partners in the health system and community agencies according to National Regulation.
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26 **Discussion**

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29 16 This study protocol presents the matched-control phase I/II clinical trial evaluating the safety and potential
30 17 efficacy of allogeneic UC-MSC administration in patients with moderate-to-severe COPD (GOLD 2019). To date,
31 18 there is no effective treatment available for COPD patients, and pharmacological interventions are hampered by the
32 19 heterogeneity of disease mechanisms and phenotypic alternation. Therefore, establishing new treatment methods to
33 20 reduce the devastating effects of COPD is imperative. The body of preclinical studies and human clinical trials
34 21 suggests that MSC administration emerges as a potential therapeutic approach for COPD because MSCs have been
35 22 found to be well tolerated and safe in many clinical trials and have proven their effectiveness in animal models. Several
36 23 clinical trials have been conducted in COPD. Most of these studies were phase 1 safety trials, which uniformly reported
37 24 no obvious adverse events and serious adverse events as well as no evidence of infusional toxicities during the follow-
38 25 up period²⁶. However, the effectiveness of MSC therapy showed differences among various clinical trials, and a small
39 26 number of trials have revealed no significant changes in lung function and fibrosis postadministration compared with
40 27 baseline levels²⁷. Therefore, it is important to comprehensively analyze the factors that directly contribute to treatment
41 28 safety and efficacy.
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48 29 Human clinical trials were conducted to evaluate the safety and efficacy of MSCs in the treatment of COPD,
49 30 including five studies using bone marrow-derived cells^{14-17 19} and a pilot study using UC-MSCs¹⁸. The first study
50 31 supported the safety profile of MSCs administered BMSCs to four COPD patients, although the overall clinical
51 32 outcomes did not demonstrate the efficacy of the treatment. It is understandable that studies together with the two
52 33 trials (NCT001110252 and NCT01306513) are phase 1 clinical trials that aimed to evaluate the safety and feasibility
53 34 of cellular administration in the treatment of COPD. Notably, the NCT001110252 study followed up with patients for
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1 up to 3 years illustrated an overall reduction in the process of COPD pathological development¹⁹. In a pilot study
2 using UC-MSCs, COPD patients were followed up for 6 months, and no AEs or SAEs were observed throughout the
3 course of the study. Although clinical outcomes such as COPD exacerbations, mMRC score, and CAT were
4 significantly reduced postadministration, pulmonary function parameters remained unchanged compared to baseline
5¹⁸. In our current study, we use UC-MSCs as an “off-the-shelf” product for administration, providing flexibility in
6 patient management and standardized products for all treated patients, allowing more accuracy in evaluation.
7 Moreover, by using a matched control design, our study aims to eliminate the variability in COPD conditions between
8 the intervention and control groups to accurately evaluate the safety and efficacy of the treatment. In general, it was
9 confirmed that MSC administration is well tolerated without serious adverse events or administration-associated
10 adverse events and is not associated with significant alterations in spirometry, immune function, cardiovascular
11 activity, or patient quality of life²⁸.

12 In both preclinical models of COPD and clinical trials, MSCs regardless of their sources exhibit their
13 therapeutic potential via either anti-inflammatory paracrine actions, reducing the rate of pulmonary fibrosis and/or
14 lung recovery. In rodent models, studies using bone marrow-derived MSCs (BM-MSCs) or adipose-derived MSCs
15 (AD-MSCs) have demonstrated that these cells administered via intravenous injection or intra-tracheal instillation
16 were safe and effective in attenuating airway injury and enhance the recovery of lung functions via reducing airway
17 inflammation and apoptosis²⁹. In mice model, administration of UC-MSCs (from Wharton’s Jelly) significantly
18 improved the pulmonary function and regeneration in COPD-induced mice compared to the sham group³⁰. To date,
19 no comparative study is conducted to address the differences in efficacy introduced by MSCs from different sources.
20 In our previous study, we demonstrated that MSCs from perinatal and adult sources behaved differently even when
21 they were cultured under a standardized culture platform (xeno-free and serum-free)³¹. Therefore, although it seems
22 that MSCs derived from different sources might show similar effects on COPD-induced animal models, we believe
23 that the source of MSCs might play a role in the level of the therapy effectiveness and their mechanism of action might
24 also differ, especially when they are exposed to COPD-related microenvironment.

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

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3 1 reduction of pulmonary fibrosis and airway thickening process, and (3) improvement of parenchymal repair by
4 2 secretion of wide range of cytokines and growth factors.

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7 3 The major delivery routes of MSCs in the treatment of pulmonary disease include intraperitoneal (usually in
8 4 animal models), intranasal or intratracheal, and IV administration. The intratracheal administration of MSCs was
9 5 performed in children with bronchopulmonary dysplasia in several small uncontrolled studies. However, in terms of
10 6 COPD, all trials utilized IV administration with the aim of investigating whether systemic administration of MSCs is
11 7 safe and effective in COPD patients. In fact, the IV administration route is considered a better option compared to
12 8 intratracheal delivery for several reasons. Previous studies illustrated that IV administration of MSCs was safe and
13 9 potentially provided therapeutic effects in several lung diseases, including COPD^{15,27}. Moreover, a systemic analysis
14 10 of preclinical studies suggested that IV administration of MSCs introduced better effects than those administered via
15 11 the intratracheal route¹³. We hypothesized that the results of this clinical trial will provide data supporting that UC-
16 12 MSC administration via the IV route is safe, feasible, and potentially effective in COPD patients.

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19 13 The dose-escalating evaluation has been conducted in several clinical trials for various diseases, including
20 14 pulmonary syndromes, using a wide range of UC-MSCs doses from 0.5 – 10 million cells/kg via IV administration¹⁵
21 15^{27,32}. Notably, limited studies have reported the different effects of MSC doses in COPD patients. In fact, a relatively
22 16 high dose (10 million cells/kg patient body weight) was tested in ARDS patients without any administration-associated
23 17 AEs or SAEs recorded. However, it is important to note that delivery of a high dose of stem cells might increase the
24 18 risk of pulmonary embolism and thrombosis regardless of administration route, which was demonstrated previously
25 19 in animal models and clinical trials³³⁻³⁵. Therefore, in this trial, we used the most common dose of MSCs used in
26 20 numerous studies, which is 1 million cells/kg patient body weight.

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29 21 In pre-clinical models of elastase-induced emphysema, two doses of MSCs improved anti-inflammatory
30 22 regulation and lung recover compared to a single dose of MSCs³⁶. In clinical trial using BM-MSCs, COPD patients
31 23 received four infusions showed a reduction in circulating C-reactive protein and in combination with lung volume
32 24 reduction strategy, two infusions were enough to increase the expression of CD31, an indication of microvascular
33 25 endothelial cell response^{17,26}. Moreover, the effects of autologous MSC administration were reported to be relatively
34 26 narrow because it was reported the positive effects in patients with type 2 diabetes were observed as early as 1 month
35 27 post-administration of MSCs and started to diminish after three to six months post-administration^{37,38}. Hence, in this
36 28 current study, we will perform two doses of UC-MSCs with a 3-month intervening interval.

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39 29 This clinical trial has several advantages. First, this is the first trial using an “off-the-shelf” product (UC-MSCs)
40 30 for COPD patients. Second, this is the first trial to investigate the therapeutic effects of UC-MSCs as supplementary
41 31 products in combination with standard medication treatments according to the GOLD 2019 recommendation. Third,
42 32 if the potential efficacy can be detected throughout the course of our study, our results (including MSC biological
43 33 analysis of stem cell characterization, immunoregulation, and metabolism) will strengthen our knowledge and
44 34 understanding of UC-MSCs effects in COPD and provide a fundamental background for treating patients with
45 35 moderate-to-severe COPD. In the case of no therapeutic effect, our data will also provide important insight into the
46 36 safety of the treatment and potential alternative approach for MSC therapy of COPD.

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5 control and UC-MSc experiment set up.

6 Contributors:

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

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14 Conflicts of Interests

15 None declared.

16 Patient consent for publication

17 Not required

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3 **1 Figure Legend:**
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5 **2 Figure 1: Schematic of the study.** COPD patients will be screened to enroll in the study. Patients from the control
6 group will be assigned to a patient from the UC-MSG group once they meet all matched criteria based on age (± 5
7 years), gender, and COPD severity classification (GOLD 2019).
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10 **5 Figure 2: Standard COPD medication treatment for both groups according to GOLD 2019 and Vietnam**
11 **Ministry of Health Guideline.** Matched COPD patients will be treated using the same treatment based on their GOLD
12 2019 classification (Groups A, B, C, and D). Group A (not included in this study): a single bronchodilator will be used
13 and based on the clinical assessments and persistence of the symptoms to continue/stop or replace by another
14 bronchodilator. Group B: Single LAMA or LABA will be initially used. If the symptoms are not reduced, a
15 combination of both LAMA and LABA will be applied. Group C: A single LAMA drug will be used for initial
16 treatment. If exacerbations occur, LAMA and LABA combination will be applied as priority. The LAMA + ICS will
17 be applied in specific cases based on clinical assessment, as the ICS has been reported to have severe side effects on
18 lung inflammation. Group D: Should start the treatment with LAMA. If the patient has CAT >20 , LABA and LAMA
19 will be used as initial treatment. LABA + ICS will be used as the initial treatment only when the patient has asthma
20 COPD overlap or the patient's eosinophil level > 300 . If exacerbation occurs after the initial treatment, the combination
21 of LAMA, LABA, and ICS should be applied. Additional roflumilast should be used if FEV1 $< 50\%$ and the patient
22 has chronic bronchitis. Macrolide should be used if the patient is a former smoker. The red arrow indicates priority
23 treatment.
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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: <ul style="list-style-type: none"> ▪ 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			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		
Medication treatment ²			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Informed consent		<input checked="" type="checkbox"/>				
Inclusion and exclusion criteria		<input checked="" type="checkbox"/>				
Demographic information		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			
Patients' medical reports		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Vital signs ³ /physical examination		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
COPD assessment	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>				
COPD GOLD 2019 classification	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Hematology analysis ⁴	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Infectious disease examination/test ⁵	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			
Blood oxygen saturation/arterial blood gas analysis ⁶	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Chest CT scan		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Chest X-ray		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Pulmonary function analysis		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Adverse event evaluation			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Mortality/complications monitoring			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

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.

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

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

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

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6: Blood gas analysis includes pH, PaO₂, PaCO₂, BE, HCO₃⁻.

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For peer review only

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)		
<i>CD73</i>		> 95%
<i>CD90</i>	Flow cytometry using the Human MSC	> 95%
<i>CD105</i>	Analysis Kit (Becton Dickinson, USA)	> 95%
Negative markers (%)		< 2%
Cell viability (%) (mean ± SD)	Trypan Blue staining	> 80%
Microorganism tests	BacT/Alert® 3D microbial detection System (Biomerieux, USA)	Negative
Mycoplasma	MycoAlert™ 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/ <i>In vitro</i> 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.

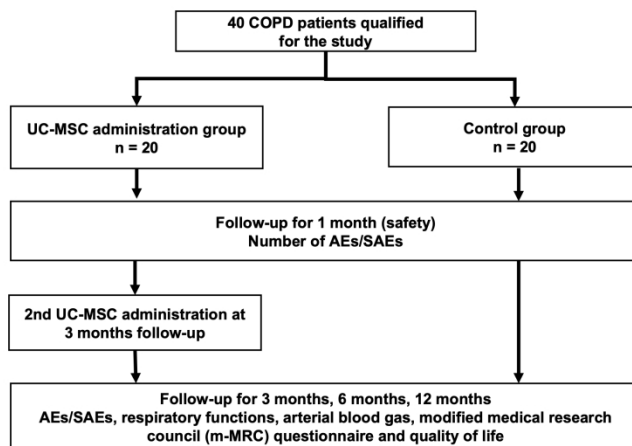


Figure 1

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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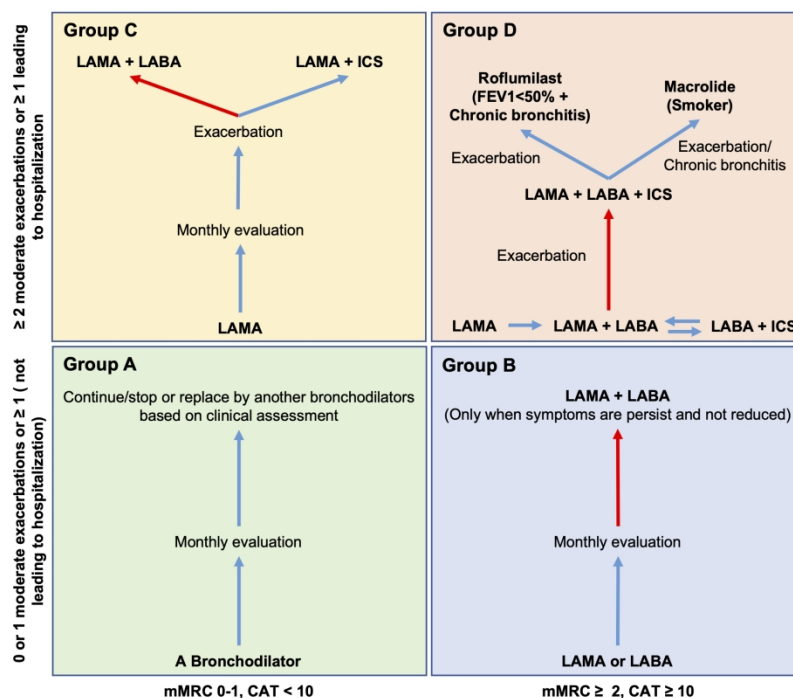


Figure 2:

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 GOLD 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

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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 SPIRIT reporting guidelines, and cite them as:

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		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

1	Roles and	#5b	Name and contact information for the trial sponsor	14
2	responsibilities:			
3	sponsor contact			
4	information			
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8	Roles and	#5c	Role of study sponsor and funders, if any, in study design;	14
9	responsibilities:		collection, management, analysis, and interpretation of data;	
10	sponsor and funder		writing of the report; and the decision to submit the report for	
11			publication, including whether they will have ultimate authority	
12			over any of these activities	
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16	Roles and	#5d	Composition, roles, and responsibilities of the coordinating	14
17	responsibilities:		centre, steering committee, endpoint adjudication committee,	
18	committees		data management team, and other individuals or groups	
19			overseeing the trial, if applicable (see Item 21a for data	
20			monitoring committee)	
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23				
24	Introduction			
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26				
27	Background and	#6a	Description of research question and justification for undertaking	4
28	rationale		the trial, including summary of relevant studies (published and	
29			unpublished) examining benefits and harms for each intervention	
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32	Background and	#6b	Explanation for choice of comparators	4-5
33	rationale: choice of			
34	comparators			
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37	Objectives	#7	Specific objectives or hypotheses	5
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40	Trial design	#8	Description of trial design including type of trial (eg, parallel	5, 6
41			group, crossover, factorial, single group), allocation ratio, and	
42			framework (eg, superiority, equivalence, non-inferiority,	
43			exploratory)	
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45				
46	Methods:			
47	Participants,			
48	interventions, and			
49	outcomes			
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53	Study setting	#9	Description of study settings (eg, community clinic, academic	5, 6
54			hospital) and list of countries where data will be collected.	
55			Reference to where list of study sites can be obtained	
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1	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
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6	Interventions:	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
7	description			
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9				
10	Interventions:	#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
11	modifications			
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15	Interventions:	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	8, 9
16	adherence			
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20	Interventions:	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8, 9
21	concomitant care			
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24	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
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34	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
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40	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
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45	Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7,8
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49	Methods: Assignment			
50	of interventions (for			
51	controlled trials)			
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54	Allocation: sequence	#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"
55	generation			
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provided in a separate document that is unavailable to those who enrol participants or assign interventions

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4	Allocation	#16b	Mechanism of implementing the allocation sequence (eg, central
5	concealment		telephone; sequentially numbered, opaque, sealed envelopes),
6			describing any steps to conceal the sequence until interventions
7	mechanism		are assigned
8			
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10			
11	Allocation:	#16c	Who will generate the allocation sequence, who will enrol
12	implementation		participants, and who will assign participants to interventions
13			
14	Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial
15			participants, care providers, outcome assessors, data analysts),
16			and how
17			
18			
19			
20	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is permissible,
21	emergency unblinding		and procedure for revealing a participant's allocated intervention
22			during the trial
23			
24			
25	Methods: Data		
26	collection,		
27	management, and		
28	analysis		
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30			
31			
32	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and
33			other trial data, including any related processes to promote data
34			quality (eg, duplicate measurements, training of assessors) and a
35			description of study instruments (eg, questionnaires, laboratory
36			tests) along with their reliability and validity, if known.
37			Reference to where data collection forms can be found, if not in
38			the protocol
39			
40			
41			
42			
43	Data collection plan:	#18b	Plans to promote participant retention and complete follow-up,
44	retention		including list of any outcome data to be collected for participants
45			who discontinue or deviate from intervention protocols
46			
47			
48	Data management	#19	Plans for data entry, coding, security, and storage, including any
49			related processes to promote data quality (eg, double data entry;
50			range checks for data values). Reference to where details of data
51			management procedures can be found, if not in the protocol
52			
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55	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary
56			outcomes. Reference to where other details of the statistical
57			analysis plan can be found, if not in the protocol
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1	Statistics: additional	#20b	Methods for any additional analyses (eg, subgroup and adjusted	10
2	analyses		analyses)	
3				
4	Statistics: analysis	#20c	Definition of analysis population relating to protocol non-	10
5	population and missing		adherence (eg, as randomised analysis), and any statistical	
6	data		methods to handle missing data (eg, multiple imputation)	
7				
8				
9				
10	Methods: Monitoring			
11				
12	Data monitoring:	#21a	Composition of data monitoring committee (DMC); summary of	10
13	formal committee		its role and reporting structure; statement of whether it is	
14			independent from the sponsor and competing interests; and	
15			reference to where further details about its charter can be found,	
16			if not in the protocol. Alternatively, an explanation of why a	
17			DMC is not needed	
18				
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20				
21				
22	Data monitoring:	#21b	Description of any interim analyses and stopping guidelines,	10
23	interim analysis		including who will have access to these interim results and make	
24			the final decision to terminate the trial	
25				
26				
27	Harms	#22	Plans for collecting, assessing, reporting, and managing solicited	9
28			and spontaneously reported adverse events and other unintended	
29			effects of trial interventions or trial conduct	
30				
31				
32				
33	Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and	9, 10
34			whether the process will be independent from investigators and	
35			the sponsor	
36				
37				
38	Ethics and			
39	dissemination			
40				
41				
42	Research ethics	#24	Plans for seeking research ethics committee / institutional review	10
43	approval		board (REC / IRB) approval	
44				
45				
46	Protocol amendments	#25	Plans for communicating important protocol modifications (eg,	10, 11
47			changes to eligibility criteria, outcomes, analyses) to relevant	
48			parties (eg, investigators, REC / IRBs, trial participants, trial	
49			registries, journals, regulators)	
50				
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52				
53	Consent or assent	#26a	Who will obtain informed consent or assent from potential trial	5, 6
54			participants or authorised surrogates, and how (see Item 32)	
55				
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1	Consent or assent:	#26b	Additional consent provisions for collection and use of	5,6
2	ancillary studies		participant data and biological specimens in ancillary studies, if	
3			applicable	
4				
5				
6	Confidentiality	#27	How personal information about potential and enrolled	10
7			participants will be collected, shared, and maintained in order to	
8			protect confidentiality before, during, and after the trial	
9				
10				
11	Declaration of interests	#28	Financial and other competing interests for principal investigators	14
12			for the overall trial and each study site	
13				
14				
15	Data access	#29	Statement of who will have access to the final trial dataset, and	10
16			disclosure of contractual agreements that limit such access for	
17			investigators	
18				
19				
20	Ancillary and post trial	#30	Provisions, if any, for ancillary and post-trial care, and for	"n/a"
21	care		compensation to those who suffer harm from trial participation	
22				
23				
24	Dissemination policy:	#31a	Plans for investigators and sponsor to communicate trial results	10
25	trial results		to participants, healthcare professionals, the public, and other	
26			relevant groups (eg, via publication, reporting in results	
27			databases, or other data sharing arrangements), including any	
28			publication restrictions	
29				
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31				
32				
33	Dissemination policy:	#31b	Authorship eligibility guidelines and any intended use of	14
34	authorship		professional writers	
35				
36				
37	Dissemination policy:	#31c	Plans, if any, for granting public access to the full protocol,	"n/a"
38	reproducible research		participant-level dataset, and statistical code	
39				
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41	Appendices			
42				
43	Informed consent	#32	Model consent form and other related documentation given to	"n/a"
44	materials		participants and authorised surrogates	
45				
46				
47	Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of	Table 2
48			biological specimens for genetic or molecular analysis in the	
49			current trial and for future use in ancillary studies, if applicable	
50				
51				

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