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Adipose-derived Stem Cells Therapy for Moderate Chronic Obstructive Pulmonary Disease in Adult Subjects: A Phase III Randomized, Multicenter Trial: RESPIRE Protocol

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Abstract

Introduction: *Chronic obstructive pulmonary disease (COPD) is one of the leading causes of death worldwide and has a prevalence of 11.7%. Current treatment focuses on reducing symptoms and exacerbations, as no definitive intervention exists. Regenerative treatments such as mesenchymal stem cell (MSCs) therapy have shown promise in preclinical and clinical studies. This article presents a study protocol to evaluate the efficacy of adipose-derived (AD) MSCs therapy in improving FEV-1 in COPD patients.*

Methods: *A phase III, multicenter, randomized, double-blind, placebo-controlled study is proposed to test the clinical safety and efficacy of AD-MSC therapy in patients with COPD. The population is subjects with COPD Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2 (50%≤FEV1<80% predicted) and Group D (two exacerbations or one hospitalization and mMRC>2 or CAT>10). This study aims to evaluate the improvement of FEV1 from baseline to 90 days after AD-MSC infusion. The primary outcome is FEV1-improvement (%) over the baseline for the treatment and placebo groups, assessed on study day 90 vs. baseline (day 0). For the primary outcome, the statistical analysis will use the Mann-Whitney U test to analyze the FEV1 for the treatment and control groups at 90 days against the baseline.*

Discussion: *Aside from symptomatic relief, there is a growing demand for a curative treatment for COPD. The potential of novel AD-MSCs therapies for COPD improvement is currently in early development. Preclinical, phase I, and a few phase II studies for AD-MSCs as a COPD treatment have proven optimistic results. This phase III, randomized, multicenter trial proposes evaluating an alternative treatment for this condition that may improve lung function.*

Introduction

Chronic obstructive pulmonary disease (COPD) was the third-leading cause of death worldwide between 2019 and 2020. (World Health Organization, 2020) With a worldwide prevalence of 11.7% and high morbidity, COPD is a significant economic problem for both developed and developing countries (Lee & Rhee, 2021).

COPD is characterized by progressive airflow obstruction mainly due to irreversible structural changes in the lung, including chronic bronchitis and pulmonary emphysema, which are associated with a chronic inflammatory reaction (Barnes et al., 2015; MacNee, 2006). The Global Initiative for Chronic Obstructive Lung Disease (GOLD) has guidelines that help diagnose and determine how advanced the disease is. The subjects are classified in GOLD groups 1 to 4, based on forced expiratory volume in one

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second (FEV1), dyspnea severity, and exacerbation history in groups A to D.

Current COPD treatment focuses on reducing symptoms and the frequency of exacerbations, as there is no definitive intervention (Vogelmeier et al., 2020). There is a demand for a curative treatment other than symptomatic relief. Nevertheless, to this point, no current therapy has proven to stop the course of the disease. An effective regenerative therapy for COPD could reduce morbidity and mortality, as well as costs in the public health system, and improve the quality of life of patients. In this light, treatment with mesenchymal stem cells (MSCs) poses a promising option, as already shown in clinical studies (Calzetta et al., 2022).

MSCs are multipotent cells that take part in the repair and regeneration of tissue (Wang et al., 2017; Fierabracci et al., 2015). In vivo research showed that MSC infusion downregulated pro-inflammatory cytokines while upregulating growth factors (Tibboel et al., 2013; Bonfield et al., 2010). MSCs can be obtained from adipose tissue or bone marrow (Dominici et al., 2006). Compared to bone marrow-derived MSCs, adipose-derived MSCs (AD-MSCs) retain their differentiation potential for longer and have a stronger capacity for immunomodulation (Melief et al., 2013). A phase II trial of AD-MSCs for COPD has demonstrated efficacy and safety (Comella et al., 2017); however, only four studies have been completed and published in the PubMed database (Antunes et al., 2017). Also, most MSC studies included only COPD subjects presenting with severe chronic lung tissue injuries that might not be reversible with stem cell therapy (Calzetta et al., 2022).

Therefore, MSC treatment for subjects with less severe lung tissue degradation has the potential to prove the regenerative benefits of MSC therapy. In addition, most studies are open-label, which could be a source of bias. Thus, a well-elaborated, double-blind, randomized, placebo-controlled trial is needed to prove the efficacy of this therapy with stem cells in COPD.

Materials and Methods

Trial Design: Parallel Arm Study

This is a phase III, randomized, multicenter, double-blind study designed to evaluate the clinical efficacy and safety of standard therapy plus AD-MSC therapy, compared with standard therapy plus placebo. The study will evaluate subjects with COPD GOLD 2 (50%$\leq$FEV1<80% predicted) and Group D (two exacerbations or one hospitalization and Modified Medical Research Council [mMRC] dyspnea scale >2 or COPD Assessment Test [CAT] >10). This superiority trial aims to evaluate the improvement of FEV1 from baseline to 90 days after AD-MSC infusion.

This study incorporates two arms: Group A, where participants receive standard therapy plus an AD-MSC IV infusion suspended in normal saline, and Group B, where participants receive standard therapy plus an IV placebo. Participants will be followed over two years.

Study Setting

This multicenter study will be conducted at three specialized centers where subjects will be identified, screened, and recruited. All sites are in the city of Sao Paulo, Brazil. A Principal Investigator (PI) will be selected at each site, who will be responsible for ensuring that the study is conducted according to the study protocol, good clinical practice, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines, and applicable local and federal regulations.

Randomization

Simple randomization will be used for this study. Separate randomization lists will be generated centrally by the clinical statistician for each participating site, and subjects will be randomized using the Signant Health System. Participants meeting the inclusion criteria for this study will be randomized with an allocation of 1:1 (verum: placebo). Randomization codes will be assigned sequentially as participants become eligible for randomization.

Allocation Concealment

An Interactive Web Response System (IWRS) via Interactive Response Technology (IRT) will be allocated to either arm, assigning a unique identification number to each eligible participant. Participants, investigators, and site staff will be blinded to treatment allocation.

Implementation

A third party will be responsible for randomization, entering subject data into the electronic system to randomly allocate subjects to the respective treatment arm. The information about randomization numbers and corresponding treatment allocation will be provided to the pharmacist in the study, who will prepare the infusion.

Blinding

Acronym	Description	Explanation
P	Population	Patients with COPD GOLD 2 (50%≤FEV1<80% predicted) and Group D (two exacerbations or one hospitalization and mMRC>2 or CAT>10) from 35 to 60 years old.
I	Intervention	SOC plus AD-MSC IV infusion suspended in normal saline
C	Comparison	Patients under the SOC and IV placebo of 0.9% saline solution
O	Outcome	*FEV1-improvement (%) baseline (day 0) vs. day 90. **FVC, FEV1/FVC, TLC, 6MWD, number of hospitalizations or deaths in 2 years, SOBDA and QOL questionnaires, BDI/TDI.
T	Time	Follow-up for 2 years

COPD: chronic obstructive pulmonary disease; **FEV1:** forced expiratory volume in one second; **GOLD:** Global Initiative for Chronic Obstructive Lung Disease; **mMRC:** Modified Medical Research Council; **CAT:** COPD Assessment Test; **AD-MSC:** Adipose tissue derived mesenchymal stem cell; **IV:** intravenous; **SOC:** standard of care; **FVC:** Forced vital capacity; **TLC:** Total Lung Capacity; **6 MWD:** 6-minute walk distance; **SOBDA:** Shortness of Breath with Daily Activities; **QOL:** quality of life; **BDI:** Baseline Dyspnea Index; **TDI:** Transition Dyspnea Index.

Table 1: *PICOT strategy for this study.*

A double-blind study design that blinds investigators, study participants, outcome assessors, statisticians, and data managers will be implemented. Blinding will be ensured during product manufacture and packaging, making AD-MSCs and placebos indistinguishable by appearance and packaging. Each treatment will have a unique barcode and package number, and it will be linked to an interactive response randomization system to give the intervention correctly. The research pharmacist will not be blinded to reassure treatment allocation. Special storage conditions are discussed in the product management section.

Bang's Blinding Index will assess blinding. The survey used in this method provides participants with 5-point scale questions ('Strongly believe the treatment is a new treatment,' 'Somewhat believe the treatment is a new treatment,' 'Somewhat believe the treatment is a placebo,' 'Strongly believe the treatment is a placebo,' or 'Do not know') regarding their perception of allocation during follow-up visits. The subject will not be notified if unblinding is observed during the survey. All unblinding events will be reported.

Emergency Unblinding

During the trial, the investigators may have to break the blinding in an emergency, especially if they think the investigational product causes a serious adverse event (SAE). Knowing about the allocation information could influence future subject care in such a case. Unblinding for specific subjects will be performed by the PI electronically through a specific procedure within the IRT. Before unblinding, the investigator should check with the institution's standard operating procedures (SOPs) and the Data Monitoring Committee (DMC) to ensure the circumstances call for it. Unblinding events will be recorded and reported in the Clinical Study Report (CSR). Participants whose treatment assignments have been unblinded by the PI will no longer continue to participate in the study but will be monitored for safety for five half-lives of the investigational product or one month, whichever is longer.

Eligibility Criteria

Inclusion Criteria:

- COPD stage GOLD 2 with GOLD D symptoms
- Ages 35 to 60
- Non-smokers or not smoking for at least the past 6 months.

Exclusion Criteria:

- History of COPD exacerbation that required hospitalization or intubation within the past 3 months.

•Current comorbidity with lung diseases such as asthma, pulmonary hypertension, tuberculosis, and other restrictive conditions.
•Active work-related exposure or secondhand smoke.
•History of clinically relevant conditions that are not controlled and not associated with COPD (heart failure, hematological, renal, hepatic, neurological, metabolic and/or autoimmune diseases, cancer).
•Incomplete COVID-19 vaccination scheme under current country recommendations.
•Alpha-1 antitrypsin deficiency.
•Body weight $\geq$150 kg or <40 kg.
•Current use of alcohol or illegal drugs.
•Pregnancy or intention to become pregnant during the study period or while breastfeeding.
•Pharmacological treatment with immunosuppressants other than the standard treatment accepted for COPD (prednisone, oral or inhaled) or other drugs under study or investigation.
•Reported allergy to any component used in the manufacture of stem cell products.
•Subjects suffering from psychiatric disorders with a commitment to judgment or behavior disorder that could compromise adherence to treatment (disorganization of actions and speeches, need for supervision for daily actions and commitments).
•Subject to previous stem cell treatments at any time in the past.

Recruitment Strategy

Convenience recruitment strategies will be used. Subjects will be recruited from external referrals (primary care, specialists' collaborations, specialized clinics, and general hospitals), specialized outpatient clinics at the host hospitals, and the clinical research center's database.

Subjects will be screened for eligibility in their first medical appointment based on inclusion and exclusion criteria. Participants will sign the informed consent if they can participate in the study and still want to after hearing about the study protocol.

Adherence

To ensure adherence, the following strategies will be implemented:

1. Educational and informative instructions at screening evaluation: Meetings or video, written or verbal information will be handed to participants to ensure they understand to the best extent what the trial aims to study and why it might be helpful for them. This will allow us to fill in any knowledge gaps subjects may have about their disease. By doing this, we can ensure the subject understands why it is essential to treat the disease and what the trial aims to help with. In this initial moment, it is important to briefly explain the study's timeline (number of visits, evaluation methods, what they are supposed to do at home, and how long they can expect the trial to last). Subjects will be given written information about the study and contact information, such as phone numbers and email addresses if they have any questions or doubts.
2. Telephone follow-up explanation: This call aims to ensure participants are comfortable participating in the trial and to answer any concerns, questions, or doubts they might have. Also, to remind the subjects of the importance of the trial and the "homework" they are supposed to fill in between face-to-face visits.
3. Homework: In the interval between face-to-face follow-up visits, to optimize the time of the subjects and favor the perception of accompaniment and thus adherence to the study, the use of a booklet or diary will be requested (see Attachment 1: Symptoms diary in the supplementary index), with a record at least weekly of doubts or concerns to comment on at the next face-to-face follow-up visit. In this document, any possible adverse effect, hospitalization, or exacerbation must also be described, including the date, time of onset, and symptoms. The delivery of the diary will be reinforced in face-to-face evaluations.
4. In-person follow-up visits: In the first month after receiving the intervention, one visit will occur every two weeks. After that, participant follow-up visits will be performed every 3 months. COVID-19 antigen tests will be performed before each visit. During the visits, measures of clinical improvement (vital signs, physical exam, 6-minute walk test) and the subjects' perception (quality of life questionnaire, adverse events report) will be assessed. In addition, complementary studies such as spirometry (at the beginning and once every three months) and CT scans (at the beginning and end of the trial and one for each exacerbation or complication) will be carried out. Expenses will be covered by the trial personnel for in-person meetings, including transportation (equivalent to public transport fare for the subject and 1 caregiver) as well as lunch for both provided inside the hospital/research facilities.
5. Keep up motivation: In face-to-face follow-up visits, doubts will be resolved, and a prudent amount of time will be given to reinforce the importance and gratitude for participation in the study, valuing the time and effort invested in attending follow-up appointments and the use of the diary as well.

Timeline	Visit 1 Inclusion	Visit 2 Infusion	Visit 3 Infusion	Visit 4 Infusion	Visit 5 Day 30	Visit 6 Day 90
Inclusion Criteria	x					
PE and MH	x	x	x	x	x	x
Intervention infusion		x	x	x		
6MWD	x					x
QOL questionnaire	x					x
SOBDA questionnaire	x					x
Baseline dyspnea index	x					x
Blood count/SGOT/ SGPT/CRP	x	x	x	x		x
COVID-19 antigen test	x	x	x	x	x	x
Check adverse events		x	x	x	x	x
Spirometry	x				x	x
B-hCG (if female)	x	x	x	x		

PE: Physical exam; **MH:** medical history; **FEV1:** forced expiratory volume in one second; **6 MWD:** 6-minute walk distance; **QOL:** quality of life; **SOBDA:** Shortness of Breath with Daily Activities; **SGOT:** serum glutamic-oxaloacetic transaminase; **SGPT:** Serum Glutamic Pyruvic Transaminase; **CRP:** c-reactive protein; **COVID-19:** Coronavirus Disease from 2019; **B-hCG:** Beta human chorionic gonadotropin

Table 2: *Timeline.*

Timeline

Details of the timeline are observed in Table 2: Timeline.

Interventions

The study drug will be purchased commercially by ATCC™ and prepared (expanded) in a laboratory setting with non-trial-related personnel to ensure blinding. The cells will be expanded in a sterile chamber to passage 5 and cryopreserved for long-term storage. To prepare AD-MSCs for therapy, aliquots of the passage 5 cells will be thawed in temperature-controlled water or an incubator on the infusion day. The AD-MSCs will be washed and suspended in a 0.9% saline solution. The cell dose will be calculated based on the participant's body weight to obtain the dose of 2 million cells per kilogram prior to the transport to the administration ward. The placebo will contain a 0.9% saline solution and be prepared on the infusion day.

Interventions will be administered intravenously, and participants will receive either an infusion containing 2 million AD-MSC/kg BW once a week for three weeks or a placebo (0.9% saline solution) infusion once a week for three weeks. Previous research showed that stem cells could not stay in the lung long before moving to the liver when given intravenously, so sequential administration was chosen. The dose was chosen based on how higher doses have proven an increased chance of reaching the target organ (i.e., the lungs) and avoiding invasive procedures like bronchoscopy (Armitage et al., 2018; Karaoz et al., 2020).

Reasons for Modification

The administration protocol can be modified in any of the following scenarios:

- In case of increased inflammation or infection biomarkers (assessed by CRP and WBC), administering one single dose can be delayed up to 7 days.
- A mild allergic reaction during administration (skin rash or pruritus without dyspnea), either self-limited or limited with a second-generation antihistamine drug, will not be considered a contraindication for the next administration but will be reported as an adverse event.
- Suppose a mild allergic reaction, as described above, happens during the first administration. In that case, the next administration can take place according to the schedule, with the administration of a prophylactic antihistaminic drug before the intervention and 2-3 days after infusion.

Reasons for Discontinuation

Discontinuation of treatment for a certain participant means that no subsequent intervention or treatment will be administered; only safety outcomes acquired after discontinuation will be considered for statistical analysis.

Discontinuation during treatment administration:

1. In case of severe allergic reaction or adverse events grade 3-5 (Common Terminology Criteria for Adverse Events, CTCAE, v5.0.), the administration will be stopped, and no further dosage will be administered. This reaction will be reported as an adverse event (AE).

Discontinuation after one administration and before the next one:
1. In case of severe allergic reactions or adverse events grade 3-5 (CTCAE v5.0), no further administration will be planned. The reaction will be reported as an AE.
2. An increase of SGOT or SGPT> 3 times the upper normal value for one of them or >2 times the upper normal value for both will lead to the discontinuation of the study. The reaction will be reported as an AE.
3. If one of the exclusion criteria is met, the subjects will be excluded (ineligible).

Discontinuation during the follow-up period:
1. If a subject is no longer eligible for the study for non-study-related reasons (e.g., if the subject has started to smoke), only safety outcomes will be assessed for this participant.
2. Ineligibility for health-related reasons, such as the development of a tumor or other systemic disease, suggests that the study be continued because establishing or not establishing a pathophysiological relationship in the context of a clinical trial is questionable, and all available data should be obtained.

No discontinuations will occur in the following scenarios:

1. AE of grade 1 and up to grade 2, according to CTCAE v5.0, are no reason for discontinuation of the study but will be observed and reported and can constitute a reason for administration delay according to the above-mentioned 7-day rule. However, all reactions will be reported as AE.

Outcomes
Primary Outcome Measure

FEV1-improvement (%) over the baseline for the intervention and placebo groups. The outcome will be assessed on study day 90 vs. baseline (day 0) for each subject. On days 0 and 90, FEV1 will be measured twice, and the average of the measurements will be used for the statistical calculation.

Secondary Outcome Measures

As secondary outcomes, the following parameters will be measured at the times (days) listed below. Each outcome will be assessed as the per-subject difference over the baseline measurement (day 0). Then, the two groups will be compared.
1. FEV1 (day 30)
2. Forced vital capacity (FVC) (day 0, 30, 60, and 90).
3. FEV1/FVC (days 0, 30, 60, and 90).
4. Total Lung Capacity (plethysmography; day 0 and 90).
5. 6 min walk distance (6MWD) (day 0 and 90).
6. Number of hospitalizations due to exacerbations over the 2-year observation period.
7. Number of deaths over the 2-year observation period.
8. Shortness of Breath with Daily Activities (SOBDA) questionnaire.
9. Baseline Dyspnea Index (BDI)/ Transition Dyspnea Index (TDI).
10. Quality of Life Questionnaire (VQ11).
10a. The questionnaires (outcomes 8–10) will be assessed on the following visits: 0 and 90.

Exploratory Outcome Measures

Blood will be collected at the suggested time points for the following outcomes and stored in the freezer. Cumulative ELISA measurements will be performed in triplicates, and the difference between the baseline (day 0) and the rest time points will be calculated per subject. Then, the two groups (treatment and placebo) will be compared—time points 0 and 90.
- Fibrinogen
- IL-6
- IL-8
- TNF-alpha
- IFN-gamma
- IL-17
- Circulating syndecan-1
- Circulating Complement C1q

Safety Assessment

Adverse events will be continuously assessed for a time frame of two years. The number (N) of adverse events between the different treatment arms will be compared for the overall time of the study (until day 90). The CTCAE v.5.0 will be used to grade adverse events in every visit and during visit intervals if needed.

Data Management

The OracleClinical® clinical data management sys-

tem will be used. Each user will only be able to use the parts of the system directly related to their job. For audit purposes, the system will also record any change in data, the user that made the change, and the time of entry or change (audit trail). Procedures will be implemented to maintain the data's integrity and confidentiality. The investigators will use electronic case report forms to enter the information into the data management system. Source data will be verified by clinical monitoring at the sites.

The data management system will do a logical check, and the data manager will do a manual check to find any mistakes in the data. Discrepancies in the data will be recorded and reviewed by the data management team, which will contact the investigators for clarification if needed. The clinical data management team will also clean the data to improve quality. After all the data has been gathered, there will be a quality check, and if no discrepancies are found, the dataset will be locked and sent to the statistician. The data will be unblinded after primary analysis. Fill out the case report forms, and the final datasets will be saved on hard drives with passwords for 15 years after the trial.

Data Monitoring

The DMC will be formed with independent experts from the research team. During the study, the members will report any possible conflicts of interest and will be able to see all of the data. The meetings will be recorded, and the DMC chair will decide how they will be run and how often they happen. When possible, the meetings will be face-to-face; if that is not possible, the committee will meet by teleconference.

The DMC will monitor subject safety, recruitment, and trial conduct. Efficacy data will also be assessed to perform a risk-benefit analysis. The committee may recommend study continuation, modification, or trial termination. The clinical data manager will develop an emergency key that gives access to the unblinded data in an emergency. The DMC chair could use the emergency key in emergencies. Any use of the emergency key will be reported.

Interim Analysis

The DMC will do the interim analysis. An interim analysis will be performed after 50% of subjects reach the primary endpoint at 90 days. At this point, the statistician will perform a statistical analysis, blinded to the group allocation. The data will be unblinded for analysis by the DMC, which will consider the risks and benefits and the costs and resources of the trial. The interim data will be restricted to the DMC. The committee could talk to the researchers about stopping or ending the trial early to avoid unnecessary procedures. The Haybittle-Peto approach will be used for superiority, with the p-value threshold set at $p<0.001$ for the interim analysis.

Sample Size Calculation

The sample size was calculated based on a similar study assessing change in FEV1 three months after treatment with AD-MSCs compared to standard treatment (Squassoni et al., 2021). The means (m1= 48.33 and m2= 43.60), delta (delta= -4.73), and standard deviations (sd1= 12.86 and sd2= 19.11) were used to calculate a sample size for this study with a significance level of 5% and a power of 80%. Stata 17 (StataCorp., 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC) was used to do the calculations, which showed that n = 376 subjects who can be evaluated are needed to answer the research question. With a 15% drop-out rate, the estimated total sample size is 444 subjects, allocating 222 subjects per treatment arm using 1:1 randomization (treatment: placebo).

Statistical Analysis For Primary And Secondary Outcomes

For primary analysis in this study, we will compare FEV1 for the treatment and control groups at 90 days against the baseline, calculating the delta between the two measurements. This will be assessed using the Mann-Whitney test. For secondary outcomes described in Section 11.2, the treatment arm will also be compared against the control group for each subject at baseline and the end of follow-up. Furthermore, paired comparison testing will compare the observations at different time points. The T-test or Wilcoxon signed-rank test will be used for continuous outcomes, and the Chi-square test will be used for categorical outcomes. Survival analysis will be assessed for the number of hospitalizations and deaths with a Cox Proportional Hazard Regression model. Normality will be determined using the Shapiro-Wilk test. P-values with an alpha of 0.05 will be used for all tests. P-values will be reported to three decimal places, with p-values less than 0.001 reported as $p<0.001$. Research questions that might come up during the study might be addressed exploratively.

Missing Data

Even though we plan to implement steps to minimize

data loss from follow-up and subjects dropping out (see Section 8), some data will be lost. As outlined above, at baseline (day 0) and after 90 days, FEV1 should have been recorded if there is no information about the primary endpoint, which is an increase in FEV1 after 90 days. The number of subjects in each group should be analyzed using the "intention-to-treat" principle, so missing data will be handled by implementing multiple imputations. Missing values will be predicted based on the information available for that subject, considering individual variability. This approach is easy to implement using the respective software and will deliver the most appropriate values. It also allows for including all subjects in data analysis. With this study design, we include secondary outcomes that will give us the information we need to do multiple imputation.

Mimputationsues due to the following reasons will be predicted using multiple imputations:
•Death of unknown cause until day 90.
•Drop-Outs due to (serious) adverse events until day 90.
•Unblinding before day 90.
•Missing information due to non-compliance of site personnel or the subject at day 90.

The reason(s) for missing values will be recorded for all subjects concerned.

Discussion

The potential of novel AD-MSCs therapies for COPD improvement is currently in early development. Preclinical, phase I, and a few phase II studies for AD-MSCs as a COPD treatment have proven optimistic results.

In animal models, MSCs therapy contributes to lung tissue repair and a better immune response to ameliorate the disease (Comella et al., 2017). Regardless of the optimistic effects on lung restoration in animal models and a phase II study that has already proven safety and efficacy (Comella et al., 2017), human analyses are based on smaller samples and focus mainly on severe presentations, which could affect the immunological mechanism of the therapy.

Aside from symptomatic relief, there is a growing demand for a healing treatment for COPD. GOLD 2 Group D subjects are clinically ill with a high risk of exacerbations but with moderate lung function. However, this population has not been considered in the current literature, which we consider important since lung damage is not severely compromised. A healing treatment becomes important before the lung gets gravely damaged, increasing mortality risk.

This phase III, randomized, multicenter trial proposes to evaluate an alternative treatment for this condition that may allow lung function improvement, covering the current literature gaps by reducing the missing translation between preclinical and clinical trials.

The study's main limitations are environmental factors (like air pollution) that could confound the result and were not adjusted for in this study design. However, this study is limited to a population-based in one big metropolitan area; thus, we expect the impact to be limited. However, future studies should be done in different countries and cities with different environmental factors to improve external generalizability.

Failure to reject the null hypothesis will suggest a lack of improvement in FEV1 after AD-MSCs therapy in the study subjects, regardless of their age and disease severity. On the other hand, if the null hypothesis is rejected, the results would conclude that the gap addressed by this study offers a novel perspective on AD-MSCs therapy, which could promote a new alternative to COPD management and a way to treat the course of the disease successfully.

Supplementary materials

Table 1: PICOT strategy for this study.
Table 2: Timeline.
Attachment 1: Symptoms diary.

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Conflicts of Interest

The authors declare no conflict of interest.

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