Allogeneic human umbilical cord-derived mesenchymal stem cells for severe bronchopulmonary dysplasia in children: study protocol for a randomized controlled trial(the MSC-BPD Trial)

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Study protocol

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Abstract

Background : Bronchopulmonary dysplasia (BPD) is a complex lung pathological lesion secondary to multiple factors, and one of the most common chronic lung diseases with poor prognosis, especially in preterm infants with severe BPD. However, there is lack of effective therapies for this disease. Stem cell therapy has been shown a promising way for improving lung injury and abnormal alveolarization, and human umbilical cord (hUC) is a good resource for mesenchymal stem cells (MSCs), which have demonstrated in some other diseases. We hypothesis that intravenous allogeneic hUC-MSCs is safe and effective for severe BPD. Methods/design: The MSC-BPD trial is a randomized single-center open-label and dose-escalation phase II trial designed to investigate the safety and efficacy of hUC-MSCs in children with severe BPD. In this study, 48 patients will be enrolled and randomly divided into two intervention groups and one control group. Patients in the intervention groups will be receive a low dose of hUC-MSCs (n=16, 2.5 million cells/kg) or a high dose of hUC-MSCs (n=16, 5 million cells/kg) in combination with traditional supportive treatments for BPD. The patients in the control group (n=16) will be treated with traditional supportive treatments alone without receiving hUC-MSCs treatment. The primary outcome measures will be the cumulative duration of oxygen therapy. Follow-up assessments will be performed at 1, 3, 6, 12, 24 months after interventions, and the key outcome during follow-up will be the chest radiography changes. Statistical analyses will evaluate the efficacy of the hUC-MScs treatment. Discussion: This study will be the first randomized controlled trial to evaluate the safety and efficacy of intravenous hUC-MSCs in children with severe BPD. Results of the trial will provide a new evidence-based therapy for severe BPD. Trial registration: ClinicalTrials.gov, NCT03601416. Registered on 26th July 2018. Key words: bronchopulmonary dysplasia, human umbilical cord-derived mesenchymal stem cells, clinical trial, protocol.

Background

Bronchopulmonary dysplasia (BPD) is a common chronic lung disease whose incidence is increasing year by year, especially at the time of two-child policy in China [1,2]. Patients with BPD usually have ventilator or oxygen dependence during early stage of the disease [3]. Most patients can gradually withdraw from the ventilator or stop oxygen treatment at different times, which depends on the severity of the disease, but abnormalities in pulmonary structure and lung function may last until early childhood even adulthood especially in those who are diagnosed as severe BPD according to the diagnostic criteria of BPD made by the National Institute of Child Health and Human Development (NICHD) [4, 5] .The mortality of overall BPD patients is about 15% [6], nevertheless, the mortality of severe BPD reaches 41%, which is an enormous threat to the health of these children [4, 7]. More than 50% survivors with BPD have experiences for hospital readmissions because of repeated lower respiratory infections in the first or two years, which cause serious economic and manpower burden to families [4, 8]. Though the surfactant treatment [9], prenatal steroid usage [1, 10], ventilator strategies [11], improved nutrition [12] are applied for BPD patients, there are still no effective therapy at present [14]. Therefore, seeking novel effective therapies to severe BPD in children is urgent and significant.

Recent years, the rapid development of stem cell technology and regenerative medicine has made stem cells as potential application for various refractory diseases, which are difficult to treat by traditional medical methods, such as degenerative diseases, cancer, and damaged tissue repair [15-17]. Mesenchymal stem cells (MSCs) are a class of adult stem cells derived from mesoderm with characters of non-tumorigenic, low immunogenicity and powerful paracrine function, which can isolate from several sources, including bone marrow, umbilical cord (UC), adipose tissue, amniotic fluid and other tissues [18, 19]. Among them, human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) are a good choice for clinical application because hUC-MSCs are easy to obtain, more proliferative and more powerful paracrine function than any other sources, and they are effective in relieving lung inflammation, fibrosis, angiogenesis and apoptosis [20-24]. Interestingly, the therapeutic potential of hUC-MSCs in several animal pulmonary diseases models, including BPD, acute lung injury, and idiopathic pulmonary fibrosis (IPF), were confirmed [22, 25, 26]. Approximately 75% hUC-MSCs are reported to be accumulated in the lungs because of the microvessel in the lung [27]. Hence, the therapeutic potential of hUC-MSCs for severe BPD in children may be effective.

To date, a multicenter, dose-escalation, phase 1 clinical trial (NCT01775774) was reported to investigate the safety and efficacy of bone marrow-derived MSCs (BM-MSCs), for moderate-to-severe ARDS in adult without any adverse events related to infusion reported, which had potential efficacy [28]. hUC-MSCs treatment was safe in patients with moderate to severe chronic obstructive pulmonary disease (COPD) (NCT00683722) [29]. Another phase I trial of 9 BPD patients in Korea was reported to be treated with human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) (NCT01297205) [30]. The follow-up data from this study showed the safety of hUCB-MSCs administration, and hUCB-MSCs could reduce the level of profibrotic factors in tracheal aspirates [31]. Despite these data indicated the safety of MSCs infusion for patients with pulmonary diseases, the limited sample size and lack of appropriate control of those trials were insufficient to show the efficacy of MSCs treatment. A large sample size of phase II trials with matched control need to further investigate the safety and efficacy of hUC-MSCs. Based on the previous promising findings, we set up a MSC-BPD trial which to evaluate the safety and the efficacy of the intravenous infusion of hUC-MSCs in chi ldren with severe BPD.

Methods

Study objectives

The goal of this clinical trial is to test the safety and efficacy of hUC-MSCs in children with severe BPD. There are three specific objectives as follow:

1. To evaluate the long-term safety and efficacy of intravenous hUC-MSCs to children with severe BPD.
2. To test the hypothesis that administration of hUC-MSCs can reduce the duration of mechanical ventilation and oxygen, and improve the impairment of pulmonary structure in children with severe BPD.
3. To explore the potential therapeutic mechanism regarding hUC-MSCs for severe BPD.

Study design and setting

The MSC-BPD trial had been registered at www.clinicaltrials.gov (NCT03601416), which is a randomized, single-center and dose-escalation phase II trial to evaluate safety and efficacy of hUC-MSCs in a total 48 children with severe BPD. A study flow chart of the trial is shown in Fig. 1. The study protocol will be reported based on SPIRIT guidelines (Additional file 1).

This trial will be conducted at Children's hospital of Chongqing Medical University (CHCMU) in Chongqing of China.

Sample size and calculation

The phase II trial is a randomized, controlled trial, and its size is calculated by power analysis using online calculator, Power and Sample Size (http://powerandsamplesize.com/). The trial is designed to investigate the hypotheses of two interventions compared to control, but not powered to test differences between the two interventions groups. The primary outcome measure is the change in the cumulative duration of oxygen therapy. The cumulative duration of oxygen therapy of severe BPD is patients reported as 90±15d [4, 13]. This trial is powered to find a difference of 15% (from 90d in the control group to 77d in the intervention groups) in participants who accept the interventions. Meanwhile, the power is set at 0.8, type I error is 0.05 and type II error β is 0.20. The calculated sample size is 14 for each group. To account for the possibility rate of patients being lost to follow-up is about 10%, the final sample size will be 16 and the total size is 48 in phase II trial.

Participants

Patient and public involvement

All the participants will not be involved in the development of the trial including the design, recruitment and conduct of the study, the selection of research question and outcome measures. The participation will be voluntary and the participants will have freedom to participate or withdraw this trial at any time throughout the study. The privacy of participants will be protected.

Participants will be enrolled into this study according to the inclusion and exclusion criteria (Table 1). The parents or guardians of all participants should provide written informed consent form approved by the Ethics Committee of Stem Cell Clinical Research of CHCMU.

Inclusion and exclusion criteria

The inclusion and exclusion criteria are listed in Tables 1. Participants' age is between 0 and 1 year old. The diagnostic criteria and severity gradation for BPD refers to the criteria established by NICHD workshop [5]. The Silverman and Andersen Score is used to access the severity of abnormal respiratory manifestations [32].

Recruitment

Patients can only be enrolled in this study after passing the citywide consultation resolution and signing the informed consent form.

There are three sources to recruit the participants. Firstly, potentially eligible hospitalized patients diagnosed as severe BPD will be informed, and asked the permission to join in this study. Secondly, patients with severe BPD who had been discharged, physicians will generate lists of patients with a diagnosis of BPD within one year in the electronic medical records from CHCMU. Investigators or physicians will contact with parents by phone or send them a research leaflet and recruitment letter. Thirdly, physicians will post study flyers at the outpatient department, the official website and WeChat public platform of CHCMU for those diagnosed as severe BPD in other hospitals. If these patients with severe BPD are interesting about this research, we will initiate the screening flow.

There will hold a multidisciplinary consultation to confirm whether these potential participants meet the general diagnostic criteria of BPD, and the inclusion and exclusion criteria. The consultation will consist of at least a neonatologist, a respiratory physician, a radiologist, a laboratory physician, an expert of Department of Critical Care Medicine (ICU), researchers of the stem cell treatment center, a staff of medical department. If more than 80% experts agree on the hUC-MSCs treatment, these patients will be viewed as the potential participants. The researcher then will arrange a meeting to communicate with the legal representative or parents about the clinical trial research detail and sign the written informed consent form.

All the matters of the clinical trial is fully explained to the guardians of patients: (1) the purpose of the study; (2) the background of the research; (3) the number of participants and duration of patient participation; (4) the procedures of the research; (5) the potential discomforts and risks of their treatment; (6) the expected benefits; (7) the protection of confidentiality and privacy; (8) the participation is voluntary. The legal representative or parents sign the informed consent after all the items above are fully understood. After that, the baseline of patients will be recorded by clinicians as listed (Table 2).

Randomization and blinding

Participants will be randomized into three groups in a 1:1:1 ratio after collecting of baseline data. The allocation sequence will be generated and sent to the investigators by a statistician. Participants will not be blinded during the phase II trial and the patients in control group will not accept the hUC-MSCs treatment.

Intervention

The hUC-MSCs produced by Ever Union Biotechnology Co. Ltd. (hereinafter referred to as "EUBIO") are transported to the ward on the day of infusion. hUC-MSCs are suspended into 0.9% normal saline. In addition to inspection the quality of hUC-MSCs product by EUBIO, the staff of the stem cell center in CHCMU confirm the cell viability and the quality of hUC-MSCs product before infusion.

There is no effective therapy for BPD patients nowadays, and these patients are often given traditional supportive treatments such as nutritional support, fluid restriction, and respiratory support (including ventilator support and oxygen supply) so that all the participants will be given with traditional supportive treatments to ensure safety. That means, the intervention groups will be given the traditional supportive treatments and extra low or high dose of hUC-MSCs infusion, meanwhile, the control group will be only given the traditional supportive treatments. Participants will be unable to use glucocorticoids 3 days before or after the hUC-MSCs treatment.

A total of 48 Patients in the intervention groups will be randomized in a 1:1:1 pattern to receive a low dose of hUC-MSCs (n=16, 2.5 million cells/kg) or a high dose of hUC-MSCs (n=16, 5 million cells/kg) in combination with traditional supportive treatments or traditional supportive treatments alone (n=16).

Withdrawal

Discontinuation may be due to participants' death, SAEs, other serious disease limiting participation or study withdrawal requested by the guardian. If the participant withdraws from the trial, the reason for the withdrawal and all the results of observations should be recorded in detail. Meanwhile, a new participant will be enrolled in the trial to replace the withdrawing subject.

Adverse events

Adverse Events (AEs) are defined as adverse medical event that occur after the subjects provide written informed consent until the end of the study visit. AEs include abnormal laboratory results, symptoms or diseases. All the AEs will be recorded in Case Report Form (CRF) and researcher should provide comprehensive clinical reports. Once AEs occur, we will follow the principle of "the first priority of the participants", take the necessary treatment according to the specific situation of the patients, and decide whether to suspend the clinical research. The main investigator should immediately inform Scientific Research Office, Medical Service, and Ethics Committee of CHCMU. The report of severe adverse events (SAEs) should be submitted in writing within 24 hours, and a follow-up report of SAEs should be submitted to Human Research Ethics Committee.

An insurance policy will be prepared for all participants in the study. They will be provided with ancillary and post-trial care for injury or death as a result of their participation in the trial.

Outcome evaluation

The outcome measures and their time frames of this trial are listed in Table 3. The primary endpoint is the cumulative duration of oxygen therapy, which means the total time of oxygen therapy from the time of starting oxygen treatment until the time of stopping oxygen treatment.

The secondary endpoints include the safety outcomes and the efficacy outcomes. The safety of the study is accessed by the number of the adverse events including SAEs, acute infusion associated adverse events (AIA-AEs) and the late infusion associated adverse events (LIA-AEs). SAEs include death,

malignant cardiac event (new ventricular tachycardia, ventricular fibrillation, or asystole, cardiac arrest), acute pulmonary embolism, stroke, anaphylactic shock, acute transplant rejection and any other diseases which extend the hospital stay. AIA-AEs include: fever, general allergic reaction (rash, edema, erythema, pale), infection of injection site, vital signs changes, laboratory test changes (the indicators of liver and kidney functions, cardiac markers, the indicators of hematology and immunity, markers of Hepatitis/Syphilis/HIV/Tuberculosis, and urinalysis, *et al.*). The LIA-AEs include tumorigenic events (tumor formation) and teratogenic events.

The efficacy endpoints are listed as follow: duration of oxygen therapy, duration of invasive mechanical ventilation, duration of noninvasive mechanical ventilation, the first time of stopping oxygen supplement, the rate of re-oxygen supplement, blood oxygen saturation, chest radiography changes, pulmonary function changes, mortality, times of hospital readmissions, complications of preterm birth.

Follow-up procedures

Follow-up assessments will be performed at 1, 3, 6, 12 and 24 months (m) after hUC-MSCs injection (Table 2). There are five times of follow-up, which will be conducted through two methods, including telephone follow-up and outpatient follow-up. The first two follow-up will be carried at 1, 3m after hUC-MSCs treatment through making a phone call to parents to ask the condition of their kid(s). The details of inquiry on the phone are shown in table 4. The next three follow-ups will be arranged at 6, 12 and 24m at the outpatient, the follow-up items are listed in table 5. The key outcome during follow-up will be the chest radiography changes.

Safety monitoring

The independent Data and Safety Monitoring Board (DSMB) will supervise the safety oversight during the trial. The members of DSMB are independent of the sponsor and trial investigators, and they have no competing interests. The DSMB will review and evaluate clinical safety and efficacy data collected according to the specified time interval in the protocol. If the the threshold of safety data exceeds a predefined threshold, the DSMB will be notified. Furthermore, the DSMB will conduct the interim analysis of all AEs occurrences during the study every 6 months. Only the data management and study designers have access to all the data in the trial. Data will be locked by the data management team when the trial completed. All the data will be provided to DSMB. If the trial terminated earlier than the expected end date, the DSMB would take part in that decision.

Data collection

The data generated during the trial will be recorded in the original medical record and CRF. The quality control personnel check the consistency of the CRF data with the original record to ensure that the data is accurately entered into the CRF. There are nine data collection points: baseline, 1d, 3d, 7d, 1m, 3m, 6m, 12m and 24m (Table 2). Within 3 days after the completion of the collection, the research records will be submitted to the research leader for review, and all these data will be submitted to the project leader

within 10 days. Next, the auditor reviews each original research record to confirm that the clinical trial data records are timely, precise, and standardized. Then, data check and entry are disposed by the statistical data manager, and data is analyzed by the statisticians.

The data of this trial will be disseminated through both national and international conferences and peer-reviewed publications. Our data set will be available after the completion of this trial.

Statistical Analysis

SPSS version 17 (SPSS Inc, Chicago, Illinois) statistical analysis software will be used to analyze the data in the study. Significant difference was determined at the α level of 0.05.

The continuous variables will be reported as means and standard deviations (SD) if the variables meet normal distribution, and t-test will be used to test the significance of difference between the two groups. Continuous variables that do not satisfied the normal distribution were expressed as X50% (X25%, X75%), and the Wilcoxon rank sum test will be used for comparison between the two groups. The categorical variables were reported as numbers (n) and percentages of the total (%), and χ2 test will be used to test the difference between two groups.

Baseline among groups will be compared using the ANOVA. The primary outcome, the duration of oxygen therapy, will be analyzed by one-way analysis of variance (ANOVA), followed by the Bonferroni test. Outcomes including the duration of mechanical ventilation, pulmonary function tests and the quantitative scores of chest radiography changes will be compared using the ANOVA. The rate including mortality, the rate of re-oxygen supplement, hospital readmission rate will be tested by χ^2 test. 24-month mortality should be analyzed by Kaplan-Meier curves.

Discussion

At present, apart from symptomatic supportive therapy, there is no effective treatment for severe BPD patients [33]. Therefore, it is imperative to seek new treatment methods to improve the prognosis of premature infants. Studies have shown that stem cell therapy can significantly improve neonatal hyperoxic lung injury [34, 35], suggesting that stem cell transplantation may be a promising way for severe BPD.

Intratracheal administration of mesenchymal stem cells in premature infants with high risk for BPD has been investigated in several small uncontrolled studies registered on the website of ClinicalTrials.gov. These studies aim to investigate whether local regional hUC-MSCs delivery in airway is safe and potentially effective, and whether this treatment could prevent BPD in premature infants. However, most patients with severe BPD are only receive the oxygen treatment while medical ventilator was no longer used. hUC-MSCs treatment through intratracheal route to those patients is difficult to operate under the circumstances. Hence, intravenous delivery method may be a better choice. Recent researches show that intravenous administration of MSCs was safe and had some potential efficacy in several lung diseases,

including acute respiratory distress syndrome (ARDS) and COPD [28, 29], but there were few reports on BPD. Based on these studies, intravenous administration of MSCs is expected to be a worthwhile infusion option for patients with severe BPD. In view of hUC-MSCs is a main source of clinical appliance, therefore, this trial aims to evaluate whether allogeneic hUC-MSCs therapy is safe and effective in severe BPD patients with a matched control. We hypothesis that this research will provide data, to show that hUC-MSCs is safe and feasible for severe BPD patients.

To date, a variety of studies on hUC-MSCs clinical trials have performed a dose-escalation of hUC-MSCs range from 0.5 million cells/kg to 5 million cells/kg in adult through intravenous infusion, and the largest dose of a few trials had reached 10 million cells/kg [28, 29, 36, 37]. However, there are still few trial reports exploring the effect of different doses of hUC-MSCs in children's diseases, especially in severe BPD patients. We will conduct a one dose-escalation trial of intravenous hUC-MSCs for the treatment of severe BPD patients. Considering that the features of premature infants with low weight, the immature function of many organs, we determine the maximum hUC-MSCs dose up to 5 million cells/kg.

Although our current trial can't perform a high quality RCT design as it's an open-label trial, there are several advantages in our trial. Firstly, this is a first trial to investigate the therapeutic effects instead of preventive effects in children with severe BPD. Secondly, this study will explore a dose-escalation of hUC-MSCs treatment through intravenous way in BPD. Thirdly, if we can observe some efficacy, the results of this study may broaden our understanding of hUC-MSCs in BPD and provide a basis for treatment for severe BPD patients. However, if there are no obvious effects, this also has important clinical implications for pediatric refractory diseases. As several kinds of patients were currently received MSCs therapy and potential efficacy were reported [30], there were lack of high credibility due to lack of randomized trials to prove safety or efficacy.

Overall, the MSC-BPD Trial is a vital and exploratory step to investigate a new evidence-based therapy for a large number of BPD patients in children.

Trial Status

Start date: July 2019.

Expected end date: December 2021.

Status at time of submission of this article: not yet recruiting.

Abbreviations

MSCs: mesenchymal stem cells; BPD: bronchopulmonary dysplasia; hUC: human umbilical cord; NICHD: National Institute of Child Health and Human Development; hUC-MSCs: human umbilical cord-derived mesenchymal stem cells; IPF: idiopathic pulmonary fibrosis; BM-MSCs: bone marrow-derived mesenchymal stem cells; COPD: chronic obstructive pulmonary disease; hUCB-MSCs: human umbilical

cord blood-derived mesenchymal stem cells; CHCMU: Children's hospital of Chongqing Medical University; ICU: Critical Care Medicine; VCANBIO: canbio Cell & Gene Engineering CORP., LTD. ; AEs: adverse Events; CRF: Case Report Form; SAEs: severe adverse events; AIA-AEs: acute infusion associated adverse events; LIA-AEs: late infusion associated adverse events; DSMB: Data and Safety Monitoring Board; SD: standard deviation; ARDS: acute respiratory distress syndrome.

Declarations

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Availability of data and materials

All the data in the trial will be available for anyone who wants to access the data following publication.

Contributors

ZF and LZ are principal investigators of this MSC-BPD trial and coordinate the operation of the entire clinical trial. JHD, LB, YS, LH, CJL, JL, XHL, KY contribute to the study design and guide the implementation of the study. XW is involved in writing the protocol, editing the manuscript. XW, YQX, OZ is involved in setting up the trial and collecting data. XHL is responsible for the integrity of the data and the data analysis.

Ethics approval and consent to participate

The study had been approved by the Ethics Committee of Stem Cell Clinical Research of the Children's Hospital of Chongqing Medical University with number 4/2018 on April 14, 2018. Participants' parents or the legal guardian(s) will be informed about the potential risks, expected benefits, rights and responsibility of the study before signing informed consent.

Consent for publication

Not applicable.

Competing interests

None declare that they have competing interests.

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Tables

Table 1. Inclusion and exclusion criteria for participants with severe BPD

Inclusion criteria

1. Participants who are male or female and whose age is 0 ~ 1 year old.

2. Participants who are diagnosed as severe BPD according to diagnostic criteria of BPD made by the National Institute of Child Health and Human Development (NICHD).[5]

3. Participants who have abnormal respiratory manifestations and the Silverman Anderson score[32] is more than 3 points.

4. Written informed consent signed by a legal representative or a parent.

Exclusion criteria

1. Participants whose age is more than 1 year old.

2. Participants who have no signs of dyspnea or BPD related changes in pulmonary imaging, such as central apnea or diaphragm paralysis although mechanical ventilation or oxygen are required.

3. Participants who have concurrent cyanotic or acyanotic congenital heart diseases, except for patent ductus arteriosus, and atrial septal defect and ventricular septal defect with defect less than 5mm.

4. Participants whose important laboratory test (liver and kidney functions teat, cardiac markers, hematology and immunity test, urinalysis etc.) abnormalities are more than three times compared with the normal value.

5. Participants who have severe pulmonary hypertension confirmed by cardiac ultrasound at the time of assessment.

6. Participants who have severe respiratory tract malformation,such as pierre-robin syndrome, tracheobronchomalacia, vascular ring syndrome, congenital tracheal stenosis, tracheo-esophageal fistula, pulmonary emphysema, pulmonary sequestration, congenital pulmonary dysplasia, congenital pulmonary cyst, congenital spasm, etc.

7. Participants who have severe chromosome anomalies (such as Edward syndrome, Patau syndrome, Down syndrome) or severe congenital malformation (such as Hydrocephalus, Encephalocele) or hereditary diseases.

8. Participants who have severe congenital infection such as Herpes Simplex, Toxoplasmosis, Rubella, Syphilis, AIDS, etc.

9. Participants who have severe active infection when CRP> 30mg / dL, or suffer sepsis or septic shock.

10. Participants who are going to have surgery within 72 hours before/after this study hUC-MSCs administration.

11. Participants who have surfactant administration within 24 hours before this hUC-MSCs administration.

12. Participants who have severe intracranial hemorrhage≥grade 3 or active pneumorrhagia or active air leak syndrome.

13. Participants who are using hormones or needing hormones within and after 7 days of hUC-MSCs administration.

14. Participants who are participating in other interventional clinical trials.

15. Participants who is inappropriate considered by the investigators or whose parents can’t afford informed consent.

Table 2. Timeline and items of evaluation during the trial

Items		Study period									
		Screening phase	Treatment phase				Follow-up phase				
		-2w	Baseline	24h	3d	7d	1m	3m	6m	12m	24m
Therapy	hUC-MSCs	√	√								
	Traditional treatment	√	√	√	√	√					
Informed consent		√									
Inclusion and exclusion criteria		√	√								
Demographic information		√									
Personal history / past history / family history		√					√	√	√	√	√
Height/weight/head circumference		√	√			√	√	√	√	√	√
Vital signs[#]/Physical examination		√	√	√	√	√			√	√	√
Hematology[$] / Blood biochemistry[%]/Urinalysis[@]		√	√	√							
Infectious Diseases related examination*		√	√	√							
Blood oxygen saturation / blood gas analysis			√	√	√	√			√	√	√
Chromosome examination		√									
Brain MRI examination		√							√	√	√
EKG		√	√	√							
Echocardiogram		√									
Ventilator parameters/oxygen therapy			√	√	√	√	√	√	√	√	√
Adverse events evaluation			√	√	√	√	√	√	√	√	√
Chest high resolution CT			√						√	√	√
Pulmonary function test			√						√	√	√
Mortality/Complications of prematurity[##]							√	√	√	√	√

#: The indicators of vital signs include temperature, blood pressure, heart rate, respiratory rate, transcutaneous oxygen saturation.

$: The condition of hematology of participants will be estimated by Hematological Tests which contains white blood cell count, platelet count, red blood cell count, hemoglobin, the percentage of lymphocytes, the percentage of neutrophil and C-reactive protein (CRP).

%: The items of blood biochemistry include liver function, renal function, cardiac markers, the indicators of immunity,and the detailed items of each test are listed as follow. Liver Function Tests: albumin, bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, prothrombin time, activated partial thromboplastin time. Renal Function Tests: creatinine, blood urea nitrogen, creatinine clearance, glomerular filtration rate. Cardiac markers: hypersensitive troponin I, CK-MBmass, myoglobin.

*: Infectious Diseases related examination include markers of Hepatitis / Syphilis / HIV / Tuberculosis.

@: The content of Urinalysis includes pH, protein, specific gravity, glucose and ketone body, white blood cells, occult blood or red blood cells, nitrite, color and turbidity.

##: Complications of prematurity include growth retardation or retardation, hearing abnormalities, retinopathy, pulmonary hypertension, left ventricular hypertrophy.

Table 3. Outcome measures

	Measures		Time Frames
Primary outcomes	Cumulative duration of oxygen therapy		Until the time of stopping oxygen therapy
	Adverse events	Number of serious adverse events	Within 24 hours post hUC-MSCs infusion
		Acute infusion associated adverse events	From the start of the trials to 1m post hUC-MSCs infusion
		Late infusion associated adverse events	Within 2 years post hUC-MSCs infusion
Secondary outcomes	The rate of supplemental oxygen therapy		At 1m post hUC-MSCs infusion
	Duration of invasive mechanical ventilation		
	Duration of noninvasive mechanical ventilation		
	The first time of stopping oxygen supplement		
	The rate of re-oxygen supplement		
	Pulmonary function changes		At the 6, 12 and 24m post hUC-MSCs infusion
	Chest radiography changes		
	Blood oxygen saturation		
	Mortality		At the 1, 3, 6, 12 and 24m post hUC-MSCs infusion
	Times of hospital readmissions		
	Complications of preterm birth		

Table 4. The telephone follow-up list

Personal information	Name:	Age:	Sex:	Date:
	Survival status:		No. ____ follow-up	
Items	Details			
Growth and development	Weight:	Height:	Head circumference:	
Numbers of upper respiratory infection			Main treatment:	
Numbers of pneumonia		Pathogen:	Main treatment:	
Numbers of readmission caused by upper respiratory infection		Length of hospital stay, days		
Oxygen therapy	☐ No	☐ Yes ____hour(s) per day		
Steroid use	☐ No	☐ Yes Kind(s) of steroid and dosage_____		
Bronchodilators	☐ No	☐ Yes Kind(s) of bronchodilators and dosage_____		
Complications of prematurity	Hearing impairment	☐ No ☐ Yes	Neurological impairment	☐ No ☐ Yes
	Vision impairment	☐ No ☐ Yes	Developmental delay	☐ No ☐ Yes
Dyspnea assessment and its grade	☐ No dyspnea			
	☐ Mild dyspnea: Only breathing faster or cyanosis when sucking milk, crying, after activity.			
	☐ Moderate dyspnea: Breathing is obviously accelerated. The Patient has shoulder lifts, three concave signs and nodding breaths. The lips are blue and irritated, but the symptoms can be improved after oxygen therapy.			
	☐ Severe dyspnea: The above performances are aggravated, and there are mouth breathing, sweating and irregular breathing, apnea, respiratory failure, often accompanied by cardiac insufficiency.			

Table 5. The outpatient follow-up list

Personal information	Name:	Age:	Sex:	Date:
	Survival status:		No. _____ follow-up	
Items	Details			
Growth and development	Weight:	Height:	Head circumference:	
Numbers of upper respiratory infection			Main treatment:	
Numbers of pneumonia		Pathogen:	Main treatment:	
Numbers of readmission caused by respiratory infection		Length of hospital stay, days		
Oxygen therapy	☐ No	☐ Yes ____hour(s) per day		
Steroid use	☐ No	☐ Yes Kind(s) of steroid and dosage_____		
Bronchodilators	☐ No	☐ Yes Kind(s) of bronchodilators and dosage_____		
Complications of prematurity	Hearing impairment	☐ No ☐ Yes	Neurological impairment	☐ No ☐ Yes
	Vision impairment	☐ No ☐ Yes	Developmental delay	☐ No ☐ Yes
Dyspnea assessment and its grade	☐ No dyspnea			
	☐ Mild dyspnea: Only breathing faster or cyanosis when sucking milk, crying, after activity.			
	☐ Moderate dyspnea: Breathing is obviously accelerated. The Patient has shoulder lifts, three concave signs and nodding breaths. The lips are blue and irritated, but the symptoms can be improved after oxygen therapy.			
	☐ Severe dyspnea: The above performances are aggravated, and there are mouth breathing, sweating and irregular breathing, apnea, respiratory failure, often accompanied by cardiac insufficiency.			
Physical examination	☐ Normal ☐ Abnormal _____			
Accessory examination	Blood gas analysis		☐ Normal ☐ Abnormal _____	
	Chest high resolution CT		☐ Normal ☐ Abnormal _____	
	Pulmonary function test		☐ Normal ☐ Abnormal _____	
	Brain MRI examination		☐ Normal ☐ Abnormal _____	

Additional File

Additional file 1: SPIRIT checklist: recommended items to address in a clinical trial protocol and related documents. (DOCX, 122 kb)

Figures

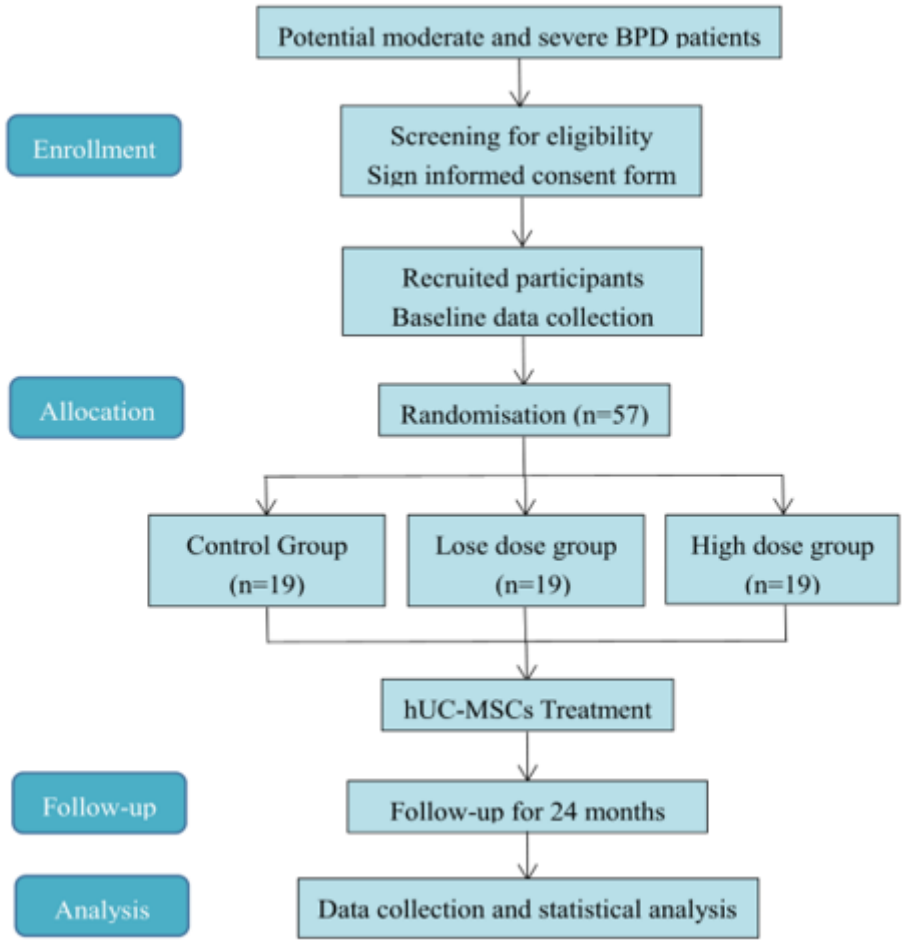

Figure 1

study flow diagram

Supplementary Files

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- Spiritchecklist.doc