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Safety and efficacy of human umbilical cord mesenchymal stem cells for the treatment of sepsis induced by pneumonia: study protocol for a single-centre, randomised single-blind parallel group trial

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ABSTRACT

Introduction Sepsis is a life-threatening organ disorder caused by a dysregulated inflammatory response to infection with no effective treatment options exist thus far. Therefore, novel therapeutic methods are urgently advocated for decreasing the high mortality rate. Recently, preclinical studies supported the efficacy of mesenchymal stem cells (MSCs) in the treatment of sepsis. In this study, we aim to test the safety, tolerability and efficacy of human umbilical cord MSCs (HUC-MSCs) for the treatment of pneumonia induced sepsis.

Methods and analysis This study is a single-centre, randomised single-blind parallel group, placebo-controlled trial. Forty eligible participants with pneumonia-induced sepsis will be randomly assigned to the observational cohort and the interventional cohort in a 1:1 ratio. In addition to the standard treatments recommended by the Sepsis 3.0 guidelines, HUC-MSCs will be administered intravenously as adjunctive therapy on day 0 at a dose of 1×10^6 cells/kg with a total volume of 100 mL diluted with normal saline through 120 mL/hour intravenous central line infusion in the interventional cohort. Placebo (normal saline) will also be administered through 120 mL/hour intravenous central line infusion at the same quantity (total volume of 100 mL) in the observational cohort. The study is approved by Research Ethics Board of East Hospital/Tongji University, which has been registered on Chinese clinical trial registry (chictr.org.cn) and initiated from October 2021. All the participants will be followed at regular intervals for 1 year. Funding is from the 'National Natural Science Foundation, China and top-level clinical discipline project of Shanghai Pudong'. This study is the first trial to assess the safety and efficacy of HUC-MSCs for the treatment of sepsis induced by pneumonia. The results will advance our understanding of the mode of action of HUC-MSCs and will also be critical for the design of future investigation in larger randomised controlled trials in multicentre. These data will offer insight into defining endpoints, key biomarkers and sample size determination.

Ethics and dissemination This study has been approved by the Research Ethics Board of East Hospital, Tongji University (Shanghai, China), which has accepted responsibility for supervising all aspects of the study (DFSC-2021(CR-04). The results of this study will be presented at both national and international conferences and be considered for publication in a peer-reviewed scientific journal. All the results presented in this study will be of group data, therefore, individual participants will not be identifiable.

Trial registration number ChiCTR2100050544, the trial is now at the stage of pre-results.

Strengths and limitations of this study

- The study is a single centre phase I, randomised placebo- ontrolled trial.
- This protocol is based on the guidance provided by the Standard Protocol Items: Recommendations for Interventional Trials 2013 statement.
- The study will adopt the sentinel method and be carried out in two stages.
- Besides the standard treatment recommended by the Sepsis 3.0 guidelines, human umbilical cord mesenchymal stem cells will be administered as adjunctive therapy on the day of inclusion in the interventional cohort.
- Clinical response will be assessed by cytokines, immune cells, serum lactic acid, and blood biochemistry as well dosage of vasopressor administration.

INTRODUCTION

Sepsis is a life-threatening organ disorder caused by a dysregulated inflammatory response to infection, which is a common clinical critical illness related to multiple organ dysfunction syndrome (MODS) and high mortality. According to surviving sepsis campaign: international guidelines for management of sepsis and septic shock

2021, current treatment is aimed at stabilising patients by fluid resuscitation, lung-protective ventilation, nutritional supply, glucose management, vasopressor support and initiation of appropriate antibiotics.[1] Despite significant advances in medications, the global mortality rate of sepsis is still up to 40% with no approved specific cell therapies.[2] In particular, pneumonia-induced sepsis is a major cause of acute respiratory distress syndrome and MODS. In previous epidemiological investigation, 33%–50% of sepsis cases were due to respiratory infections, 25%–32% to genitourinary infections, 11%–23% to a gastrointestinal source,~7% to a bone or joint infection, 5%–11% to a skin or soft tissue infection, and 3% to other sources; 3% of infections involved more than one source.[2] Moreover, pneumonia induced sepsis has caused about 40%–60% mortality.[3] Therefore, it is highly urgent to develop new therapeutic methods for sepsis especially originated from pneumonia.

Mesenchymal stem cells (MSCs) can be derived from a variety of tissues, such as adipose tissue, lung, liver, skeletal and heart muscle, amniotic fluid, synovial membrane, placenta, dental pulp and umbilical cord blood.[4] Large number of studies have documented the immunomodulatory, antiapoptotic, anti-inflammatory, tissue repair, angiogenesis and microbial clearance properties of MSCs in various diseases.[4–6] Recent studies had documented the novel ability of MSC to release paracrine factors, secrete exosomes and microvesicles and transfer mitochondria.[7 8] Hence, the application of MSCs may become a good candidate for the treatment of sepsis (the molecular mechanism is summarised in figure 1). In preclinical studies in mouse models of peritoneal sepsis, including models that used endotoxin or live bacteria, the administration of MSCs had shown positive outcomes with reduced expressions of proinflammatory factors, promoted bacterial clearance as well as alleviated the injury and improved the dysfunction of the different vital organs, including heart, lungs, kidney and liver.[9] Although the murine experiments had provided considerable support for potential clinical applications in sepsis, they still do not completely simulate the heterogeneity and complexity of the clinical conditions.

Bone marrow (BM) was the first reported source of MSCs. However, BM is not suitable for clinical use for its highly invasive donation procedure and the decline in differentiation potential with culture time.[10] Human umbilical cord MSC (HUC-MSCs) are derived from the umbilical cord tissue of perinatal fetuses, which belong to a relatively primitive stem cell population with wide availability.[11] HUC-MSCs are easy and convenient to obtain as well as have a small chance of infection with virus. They have no adverse effects on donors or recipients and not subject to ethical restrictions. After multiple proliferation, the cell morphology, biological characteristics, anchorage dependence, contact inhibition and serum dependence remain stable. Moreover, they provide a distinct advantage due to their lack of MHC II antigens, thereby providing an off-the-shelf, allogeneic therapy.[12–14] Hence, they are thought to be more suited for clinical applications and a possible extra-embryonic MSC source for cell-based therapies. Until now, very few clinical trials have been performed on the therapeutic potential of HUC-MSCs to control inflammation and reduce tissue damage caused by sepsis and septic shock in the clinical setting. A total of 6 clinical trials had been registered on ClinicalTrials.gov to adopt MSCs for therapy of sepsis syndrome, among which three trials had been finished. However, MSCs used in these trials were derived either from BM or adipose tissue.[15] Only a phase I trial in China registered on chictr.org.cn (ChiCTRTRC14005094) had reported the safety and efficacy of HUC-MSCs transplantation in severe sepsis patients.[16] However, the sample size of that study was very small with only 15 patients enrolled and divided into three dosage groups (each group with only 5 patients). Most importantly, it was not a prospective study which set a historical case-matched comparison group as the control. Furthermore, sepsis is a highly heterogeneous syndrome and the diversity of aetiology will confound the interpretation of clinical study. To our knowledge, no previous clinical study had been conducted on septic patients caused by the same aetiology. Herein, we aim to describe the study design and methodology of this single-centre, randomised single-blind parallel group trial to assess the safety, tolerability, and efficacy of HUC-MSCs as adjunctive therapy in patients with pneumonia induced sepsis.

Objectives

The primary objective is to determine the safety profile of central line infusion of HUC-MSCs administered at a dose of 1×10^6 cells/kg by monitoring any serious adverse events (SAEs) or AEs and potential immunological host responses against the administered cells during the follow-up period. The secondary objective is to explore the clinical efficacy of HUC-MSCs in terms of improvement of Sequential Organ Failure Assessment (SOFA) score and Acute Physiology A-and Chronic Health Evaluation II score, survival/clinical cure as well as other efficacy-related endpoints. A further objective is to understand the mode of action of HUC-MSCs in patients with sepsis induced by pneumonia by identifying the proinflammatory and anti-inflammatory pathways through which HUC-MSCs may affect the underlying processes of sepsis.

METHODS AND ANALYSIS

Study methods

This study is a single-centre, randomised single-blind parallel group, placebo-controlled trial. We will adopt

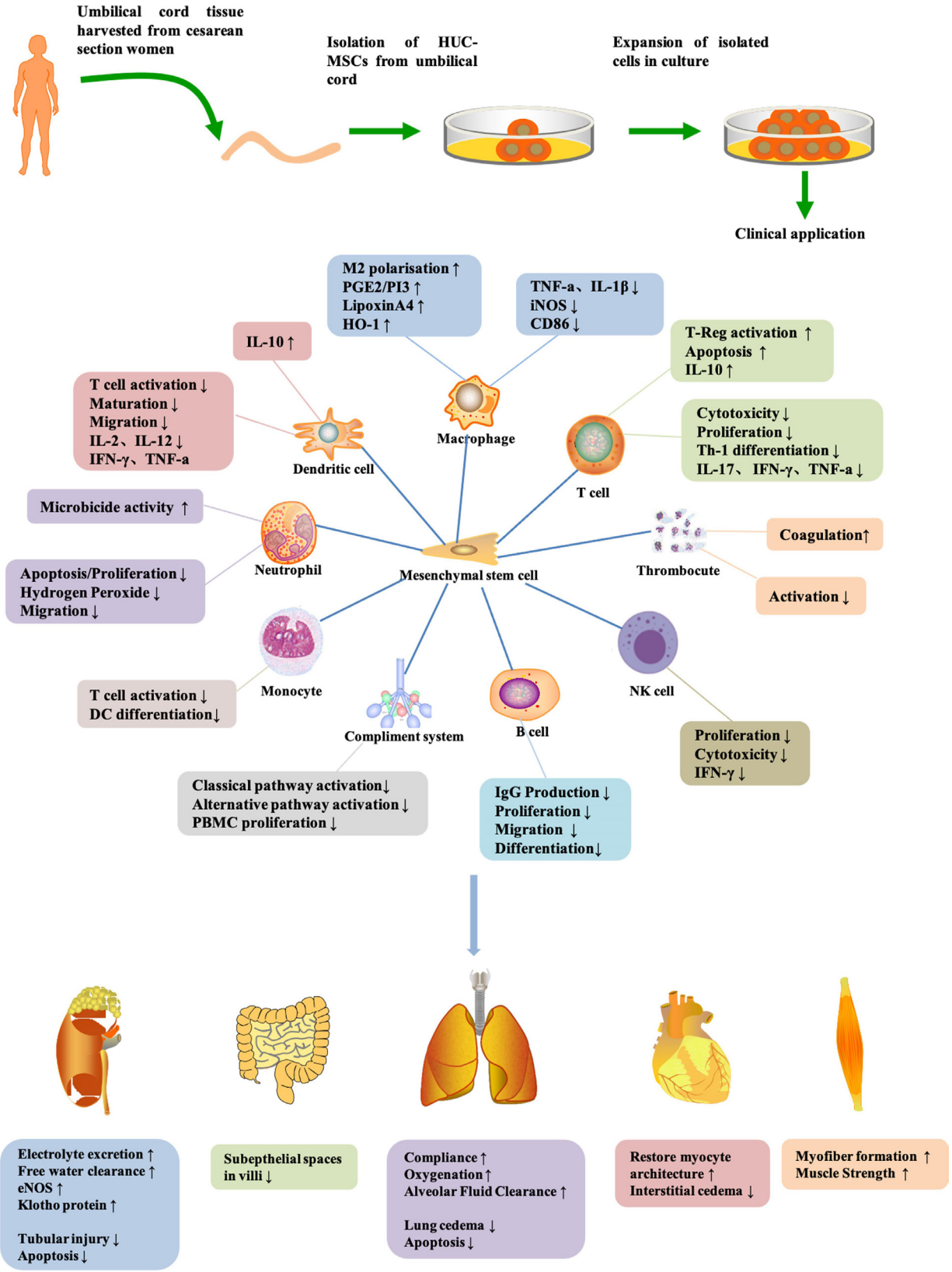

Figure 1 Umbilical cord mesenchymal stem cells (MSCs) are sampled from caesarean section woman and expanded ex vivo. Their immunomodulatory capacity are used for the treatment of sepsis. HUC-MSCs modulate inflammation through the generation of regulatory immune by reducing proinflammatory cytokines; increasing the release of the anti-inflammatory cytokine, inhibiting apoptosis of immune cells and reducing lymphocyte, neutrophil and macrophages infiltration. HUC-MSCs also have antimicrobial effects as they increase the phagocytic capacity of monocytes, macrophages and neutrophils. Due to these properties, HUC-MSCs can reduce organ injury and increase functionality, thus conferring a theraputic benefit. DC, dendritic cell; eNOS, endothelial nitric oxide synthase; HUC, human umbilical cord; PBMC, peripheral blood monoculear cell.

the sentinel method and carry out in two stages. The sentinel group will enrol six cases in each cohort to observe the safety for 3 months. Afterwards, 14 subsequent cases will be enrolled. Finally, a total of 40 eligible participants will be randomly assigned to the observational cohort and the interventional cohort in a 1:1 ratio. Besides the standard treatment recommended by the surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021 on the day of inclusion, HUC-MSCs will be administered as adjunctive therapy at a dose of 1×10^6 cells/kg with a total volume of 100 mL diluted with normal saline (NS) through 120 mL/hr intravenous central line infusion in the interventional cohort.[1] Placebo (NS) will also be administered through 120 mL/hour intravenous central line infusion at the same quantity (total volume of 100 mL) in the observational cohort. The study was initiated in October 2021 and the participants will be followed at regular intervals for 1 year if possible.

Study design

This protocol is based on the guidance provided by the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 statement (figure 2).[17] A SPIRIT schedule of enrolment, interventions and assessment is provided in figure 3. Our study is a single-centre, randomised single-blind parallel group, placebo-controlled trial that planned to enrol 40 patients with sepsis induced by pneumonia. The study will be conducted from October 2021 to September 2024 in Shanghai East Hospital/Tongji University. Once eligibility is confirmed, subjects will receive standard-of-care (Soc) according to the 2021 surviving sepsis campaign guidelines plus 100 mL intravenous central line infusion of HUC-MSCs

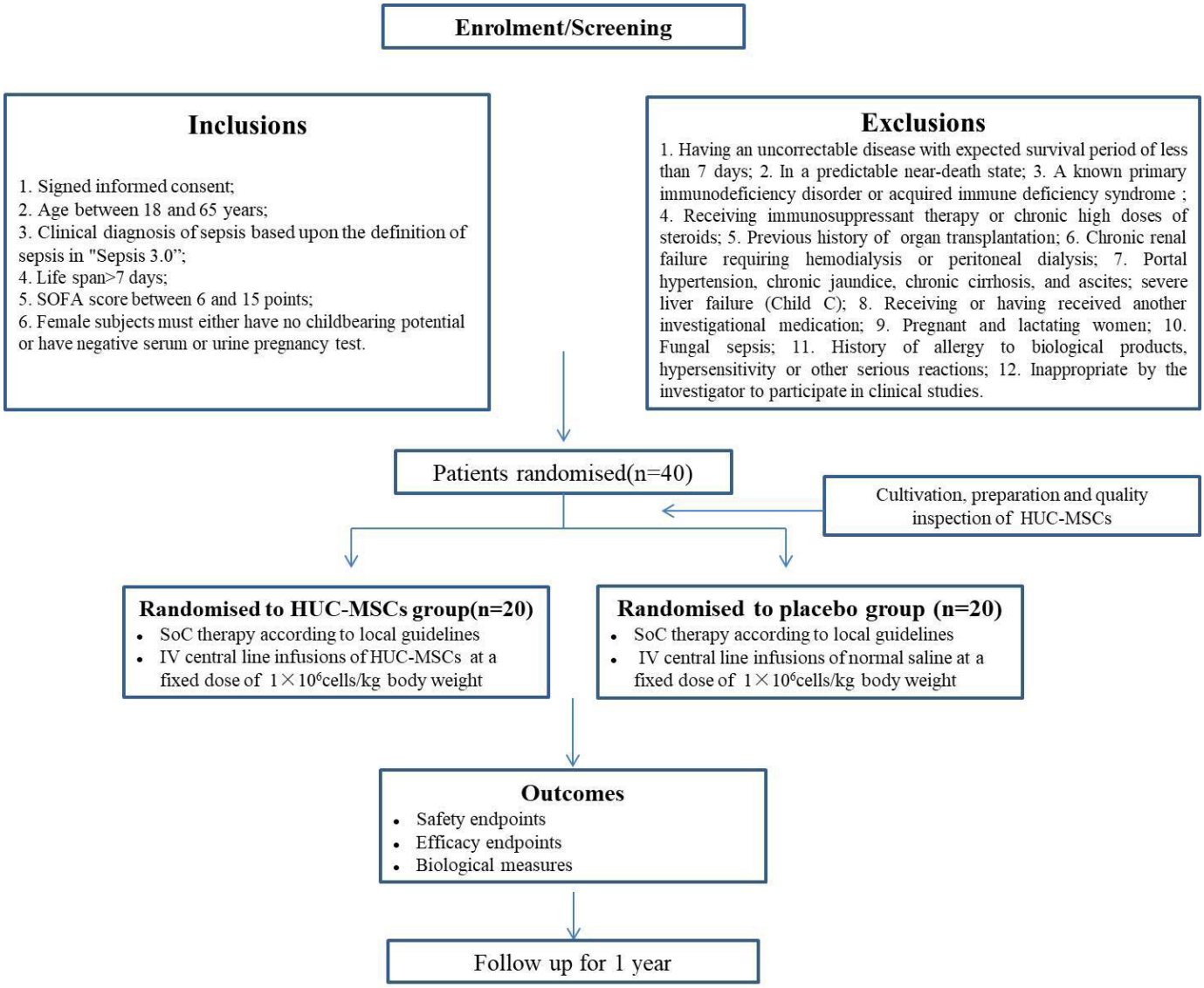

Figure 2 The schematic diagram of the trial design. A single-centre, randomised single-blind parallel group trial to assess the safety and efficacy of human umbilical cord (HUC) mesenchymal stem cells (MSCs) for the treatment of sepsis induced by pneumonia. IV, intravenous; Soc, standard of care; SOFA, Sequential Organ Failure Assessment.

	Enrolment	Intervention	Study phases										
Visits	/	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11
Time-point	D-1	D0	D1	D3	D7	D14	D21	D30	D60	D90	D180	D270	D360
Time deviation	± (1d)							± (3d)					
Inclusion/ Exclusion criteria	X												
Informed consent	X												
Medical history collection: age, gender, height, weight, comorbid diseases, smoking history, family history, current illness history, etc.	X												
Current symptoms	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination, vital signs	X	X	X	X	X								X
Blood routine	X		X	X	X	X	X	X	X	X	X	X	X
Urine routine	X		X	X	X								X
Stool routine	X		X	X	X								X
Blood biochemistry/Blood sugar/Blood lipid	X		X	X	X	X	X	X	X	X	X	X	X
Myocardial injury markers	X		X	X	X	X	X	X	X	X	X	X	X
NT-proBNP	X		X	X	X	X	X	X	X	X	X	X	X
Coagulation function index (including D-dimer)	X		X	X	X	X	X	X	X	X	X	X	X
Blood gas analysis	X		X	X	X	X			X		X		X
Infection-related indicators [1]	X		X	X	X	X			X		X		X
Cytokines[2]	X		X	X	X	X			X		X		X
Immune cells related [3]	X		X	X	X	X			X		X		X
Tumor index	X		X	X	X	X	X	X	X	X	X	X	X
HIV screening	X												
18-lead ECG	X		X	X	X	X	X		X		X	X	X
Abdominal Color Doppler Ultrasound	X												
Chest CT examination	X								X		X		X
APACH II score	X		X	X	X								X
SOFA score	X		X	X	X								X
Combination therapy	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events record		X	X	X	X	X	X	X	X	X	X	X	X

Figure 3 Schedule of enrolment, interventions and assessments. 1. Infection-related indicators: CRP, PCT, SAA, HBP, LL-37. 2. Cytokines: IL-1β, IL-6, TNF-a, IL-17A, IL-2R, IL-8, IL-10, NF-κB, TGF-β.3. Immune cells related: CD3, CD4, CD19, CD8, CD25, NK, CD14, HLA-DR, DC. APACHII, Acute Physiology A-and Chronic Health Evaluation; NT-pro-BNP, N-terminal forebrain natriuretic peptide; SOFA, Sequential Organ Failure Assessment;.

at a fixed dose of 1×10^6 cells/kg (120 mL/hour), or placebo (100 mL intravenous central line infusion (120 mL/hour) of NS).[1] The randomisation and the infusion of HUC-MSCs or placebo will be performed as early as possible within the first 12 hours of patients fulfilling the criterion. The day of administration of HUC-MSCs (or placebo) will be considered day 0 of the study. The maximum screening duration will be 12 hours and treatment duration will be 1 day. The study will permit concomitant Soc, including antibiotic and other therapies, in an add-on design. Figure 2 shows the schematic diagram of the trial design.

Setting

The project takes place in the Department of Internal Emergency Medicine and Critical Care, Shanghai East Hospital, Tongji University School of Medicine. More than 2000 critically ill patients are admitted each year, among which over 300 suffering from sepsis. The incidence of sepsis induced by pneumonia is 45%–61%

in our hospital. Therefore, the inclusion of qualified cases can be guaranteed.

Study participants

The reference population will consist of adult patients admitted to the Emergency Department with sepsis. Eligible patients will include those with clinical diagnosis of sepsis induced by pneumonia.

Patients and public involvement

Although patients were not involved in the development, planning, recruitment, conduction or burden assessment of this study, the study concept was approved by the Research Ethics Board of East Hospital, Tongji University (Shanghai, China).

Participant eligibility

Inclusion criteria

1. Signed informed consent provided by the patients (or relatives or legal representatives). The informed consent form includes information that data will be recorded, collected, and processed.
2. Age between 18 and 65 years.
3. Clinical diagnosis of pneumonia (online supplemental material 1) induced sepsis based on both the definition of pneumonia and sepsis in 'Sepsis 3.0',[1 18] namely infection and SOFA score ≥2 points.
4. Survival time >7 days.
5. SOFA score between 6 and 15 points.
6. Female subjects must either have no childbearing potential or have negative serum or urine pregnancy test.
7. Sexually active subjects (of both sexes) must agree to use contraception for the entire duration of the study, or for 3 months after the investigational medicinal product.

Exclusion criteria

1. Having an uncorrectable disease, such as advanced tumour or other advanced disease, resulting in an expected survival period of less than 7 days.
2. In a predictable near-death state.
3. A known primary immunodeficiency disorder or acquired immune deficiency syndrome with CD4 counts less than 50 cells/mm3 or not receiving highly active antiretroviral therapy.
4. Receiving immunosuppressant therapy or chronic high doses of steroids.
5. Previous history of BM lung, liver, pancreas or small bowel transplantation.
6. Chronic renal failure requiring haemodialysis or peritoneal dialysis.
7. Portal hypertension, chronic jaundice, chronic cirrhosis, and ascites; severe liver failure (Child C).
8. Receiving or having received another investigational medication within 30 days prior to the start of the study.
9. Pregnant and lactating women.
10. Fungal sepsis.
11. History of allergy to biological products, hypersensitivity or other serious reactions.
12. Subjects deemed inappropriate by the investigator to participate in clinical studies.

Interventions

Patients receive HUC-MSC transplantation or placebo as assignments. HUC-MSCs will be dispersed and diluted in 100 mL of NS. A quick check will be performed to ensure the appropriate cell count ($5.5–6.5 \times 10^7$) and viability rate (> 90%) before transplantation. Then transplantation will be completed through 120 mL/hour intravenous central line infusion in the interventional cohort. Placebo will be prepared by 100 mL of NS and given in the same process as HUC-MSC transplantation in the observational cohort. All the subjects will follow the same schedule than the active treatment (online supplemental material 2).

Follow-up

All patients will be followed up at regular intervals for 1 year if possible.

Withdrawal

In accordance with the Declaration of Helsinki, patients or their legally authorised representatives shall have the right to withdraw at any time for any reason specified or unspecified. Withdrawal from the study will not affect the patients' further treatment or care. However, the data collected prior to their withdrawal treatment will be retained and used in the analysis, unless they explicitly revoke permission to retain the data. The reason for discontinuation will be recorded in the case report form (CRF). The criteria for termination and withdrawal are as follows: unblinding; occurrence of AEs that justify the withdrawal from the study; major protocol deviations; withdrawing informed consent; being pregnant during treatment. Any identified AEs will be followed until resolution.

Outcome measures

The safety endpoints assessed throughout the study will be involved in the incidence of treatment-emergent AEs, including hypotension, haemorrhage, acute coronary syndrome, tachyarrhythmia, pulmonary embolism, allergic reaction or anaphylactic shock. During this process, Safety Monitoring Committee will be involved to monitorise safety and to propose changes in the protocol (table 1).

Changes in vital signs will be assessed from screening, 1 hour (both before and post-dose) and every 2 hours postdose in the first 24 hours. The participants will be followed at regular intervals for 1 year. All AEs will be judged as being related or not to the study treatment.

Efficacy endpoints measured will include SOFA score over 7 days, survival at day 28 after treatment as well as average survival time. Clinical response will be assessed by cytokines, immune cells, serum lactic acid, blood biochemistry as well dosage of vasopressor administration.

Table 1 Data collection for the study

Demographic and baseline data	Safety data	Efficacy data	Biological data
► Age ► Gender ► Height ► Weight ► Smoking history ► Family history ► Medical history and prior medication taken within 2 weeks before the inclusion in the study ► All patients will experience a complete physical examination at screening	► All SAE or AE, including hypotension, haemorrhage, acute coronary syndrome, tachyarrhythmia, pulmonary embolism, allergic reaction or anaphylactic shock. ► Vital signs, including temperature, MAP, HR, SPO2, PaO2/FiO2 ► Physical examination ► 12-lead ECG ► Laboratory safety assessments	► SOFA score ► Mean survival time ► Vasopressor treatment ► Laboratory efficacy assessments ► Survival at day28 after treatment	► Factors related to infection, such as blood routine, CRP, PCT, SAA, HBP, antibacterial peptide LL-37 ► Cytokines, ► Immune cells ► Serum lactic acid ► Blood biochemical indicators

AE, adverse event; APACHE, Acute Physiology A-and Chronic Health Evaluation; CRP, C reactive protein; HBP, Heparin-Binding Protein; HR, heart rate; MAP, mean artery pressure; PCT, procalcitonin; SAA, serum amyloid A protein; SAE, Serious Adverse Event; SOFA, Sepsis related Organ Failure Assessment.

Participant timeline

Once a suitable patient has been identified, the maximum screening duration will be 12 hours after signing informed consent. However, initiation of treatment with HUC-MSCs /placebo should be performed as early as possible within this 12 hours window. The treatment period (day 0) starts with the administration of the first HUC-MSCs/placebo dose and ends with the end of the infusion.

Sample size

Our study is an exploratory randomised controlled study. Therefore, no hypothesis testing comparing outcomes between treatment arms has been obtained. We decided the sample size according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use E9 guideline (ICH E9 R1).[19] As little is presently known about the outcome measures used in the study of HUC-MSCs, our study will first provide the clinical dataset necessary for determining endpoints and enable preliminary estimates of effect size for the design of future studies of HUC-MSCs used in patients with sepsis induced by pneumonia.

Recruitment

Since patients with sepsis induced by pneumonia require prompt treatment, study enrollment will take place during a short time window (up to 12 hours), starting from when the patient is first identified to fulfil the appropriate severity criteria and ending with the administration of HUC-MSCs. We will keep a log of all screened patients with sepsis induced by pneumonia, and investigators will record the reasons for not including patients who were screened but not enrolled.

Randomisation and allocation

This study uses the principle of minimal randomisation to implement central randomisation allocation. After performing the screening visit and verifying subject's eligibility by inclusion and exclusion criteria, investigators will place a screening and log into the randomisation system. After finishing the screening information, the system will provide a randomisation authorisation code, which will allow the patient to be randomised within the study. Codes in a random sequence are assigned to patients by the treatment team without knowing that each code is in the interventional or observational group. Patient codes are then matched to randomly generated sequence information for interventions.

Blinding

All participants are unaware of which group of this study they are in. The lead researcher, care givers, data collectors and outcome assessors are aware of the grouping of patients. They will not be permitted to share information about the treatment with any member of the blinded team. To ensure blinding, the primary packaging of HUC-MSCs and placebo will be identical. Additionally, a specific blinding plan will document all personnel involved in the trial and their responsibilities.

Data collection and management

Data management will be performed according to the study-specific Data Management Plan in accordance with International Conference on Harmonisation guidelines and clinical research organisation standard operating procedures (SOPs). The study data is stored in the Shanghai East Hospital in accordance with relevant data privacy regulations. Data entry, validation and data queries will be handled using the Raw Data Sheet and the

CRF. All cases will be observed according to the above plan, and the case record form will be filled carefully. The patient's medication status will be carefully recorded and explained in detail. Various data in clinical research should be recorded, and various laboratory reports should be pasted on the medical record sheet. Patient information during the study must be recorded in the CRFs in an anonymous form, identified just by the patient number and the initials of the phonetic alphabet.

Before database closure, reconciliation will be performed between the SAEs entered in the safety database and the study database. Any deviations, that is, discrepancies and additions from the process defined in the Data Management Plan, will be described in a study-specific data management report. When data for the primary endpoint are available and before database lock, a blinded adjudication committee will review subject evaluability, sepsis induced by pneumonia clinical response assessment and patient assignment processes. Access to the data is only available to the personnel participating in the study, no information is provided to non-examiners. For computer analysis, all personal data are encoded so that patients are unidentifiable when processing or reporting the results.

If the CRF needs to be modified, the researchers must keep track of the changes. Modifications should be approved and signed by the researcher and, if necessary, the reason for the modification should be mentioned. The research auditor will review the completeness and accuracy of the CRF and guide the testers in making the required corrections and additions.

General statistical methods

The occurrence of all incidences of infusion-associated events as well as SAEs, including death, and non-SAEs thought to be related to the HUC-MSC infusion will be evaluated. Dichotomous data will be presented with proportions and 95% CIs. For continuous variables, we will use means and SDs to present normally distributed data; for other data, we will use medians and IQRs. Numbers and percentages will be used for categorical variables. Continuous variables will be compared using the t-test or Mann-Whitney test, categorical variables using the χ^2 test or Fisher's exact test. Baseline and on-study SOFA scores among groups were compared using an analysis of variance. Repeated Measures Anova will be used to detect mean differences in the independent variables of treatment and time. Cox proportional hazards regression will be performed to screen the potential safety endpoints. Kaplan-Meier curve will be used to assess the prognostic values of the efficacy endpoints. Individual patient data will be listed. The results of all laboratory test result, physical examination findings, ECGs and vital signs will be presented in data listings; safety laboratory data will be presented by absolute and changes from baseline values by visit. SPSS V.17 was used for all statistical analyses. All abnormalities will be assessed for potential clinical relevance. All of the analyses performed in this study will be considered statistically significant at a level of 5%, and 95% CIs will be calculated for all of the data.

Monitoring and auditing

The main responsibility of the inspector is to supervise the research process, to ensure that the research follows the plan and comply with the GCP principles, to ensure that the research records and reports are accurate and complete, and to confirm that all subjects have informed consent before entering the research. If there is any inconsistency with the plan during the research process, the inspector should report to the sponsor and the ethics committee in a timely manner.

All clinical research data and documents should be verified; all observations and research findings should be verified to ensure the credibility of the data. Quality control is adopted at each stage of clinical research to ensure reliable data and correct research process. And data management and quality control are in accordance with data management and quality control SOPs.

Ancillary and post-trial care

The researcher keeps all the data in the database of Shanghai East Hospital, Tongji University School of Medicine in China, including the confirmation of all participating subjects (which can effectively check different records, such as the original records in the hospital), all original informed consent forms with patient signatures, all observational forms, detailed records of distribution, etc. The investigator should keep the clinical research data until 5 years after the termination of the clinical research work; the sponsor should keep the clinical research data permanently.

Protocol amendments

Important protocol modifications like changes in eligibility criteria or outcome will be communicated to the relevant parties, that is, sponsor, trial registry and scientific ethical committee.

Confidentiality

All study-related information and participant information will be stored securely at the study site in areas with limited access. Password-protected access systems will be used to ensure the confidentiality of local databases.

ETHICS AND DISSEMINATION

Ethics approval

The protocol for this study has been approved by the Research Ethics Board of East Hospital, Tongji University (Shanghai, China), which has accepted responsibility for supervising all aspects of the study (DFSC-2021(CR)-04). Written informed consent to participate in the study must be obtained from the patients or their legally acceptable representative, as required by national laws, respective regulations and institutional review boards/independent ethics committees/regional ethics boards. Consent will be collected using a short form provided in Simplified

Chinese (or in other languages on request). The form includes a description of the study, reasonably foreseeable risks or discomfort to the participant, and the rights of the participant, including withdrawal of participation at any time. The form may be signed via signature or thumb print. Oral interpretation of the consent form may be provided for illiterate participants. Translators involved in consent taking will also be asked to sign the form. A copy of the form will be provided (paper and/or electronically) to the participant on signage for the participant to keep as a record. This study has been registered on Chinese clinical trial registry (www.chictr.org.cn). Only the researchers associated with the study and the Ethics Committee would have access to the research data.

Dissemination policy

The results of this study will be presented at both national and international conferences and be considered for publication in a peer-reviewed scientific journal. Positive, negative and inconclusive results will be published.All the results presented in this study will be of group data, therefore, individual participants will not be identifiable.

The list of authors of the publication will be defined according to International Committee of Medical Journal Editors criteria, involvement in trial design, oversight, number of evaluable patients enrolled, analysis and interpretation of data, and preparation of manuscript. The study will only be published once it has been finished and the final analysis is completed; the final manuscript must be approved by all the authors before publication. Medical writing support will be used as required.

DISCUSSION

With the ageing of the population and the increase of invasive medical treatments, the incidence of sepsis continues to rise.[20] Millions of septic patients worldwide increasing yearly, of which more than 40% die, making it the leading cause of death in intensive care units. To date, no definitive treatment for sepsis has been introduced, novel therapies are urgently needed for controlling the inflammation and organ dysfunction caused by sepsis. Animal studies have confirmed the potential value and mechanism of HUC-MSCs to control the inflammation, regenerate the damaged tissues/organs and promote the bacterial clearance in sepsis.[21–24] It is urgent for clinical trials to evaluate the safety and efficacy of HUC-MSCs in the treatment of sepsis caused by the same aetiology before they can be used effectively. To our knowledge, this is the first trial to assess the effects of HUC-MSCs in pneumonia induced sepsis.

The first phase I clinical trial was started in 2011 to examine the safety and efficacy of allogeneic MSCs in the treatment of neonatal sepsis. In that study, nine infants were treated with allogenic HUC-MSCs and monitored for 24 months (in three-time intervals, including 4–6 months, 8–12 months and 18–24 months). The results showed that HUC-MSCs transplantation may be a safe and effective method to treat neonatal sepsis.[25] Another result from a randomised controlled phase I dose-escalation trial of BM-derived MSCs in septic shock in Canada (NCT02421484) also suggested that doses up to 250 million cells may be safe and well-tolerated.[26] More recently, a single-centre randomised controlled phase I clinical trial in China (ChiCTRTRC14005094) was conducted and reported that a single-dose allogeneic HUC-MSCs infusion of up to 3×10^6/kg was safe and well tolerated, with no SAEs related to MSC administration after 18 months of follow-up. The team is now conducting a randomised, double-blind, placebo-controlled phase II clinical trial in 70 patients with severe sepsis by infusing HUC-MSCs at a dose of 3×10^6 /kg, with the aim of focusing on its safety and secondary results including survival rates and systemic endpoints.[16] Besides, another phase II trial is currently ongoing to assess the efficacy and safety of intravenous infusion of 300 million allogeneic, BM derived MSCs in the treatment of septic shock (NCT03369275).

However, there are a few issues concerning the abovementioned clinical trials which may impact the translation of MSCs therapy to the clinical setting. First, the aetiology of sepsis was no single origin and might lead to an increase in confounding factors and difficult to evaluate effects. Second, the limitation of sample size may further be amplified by the complex comorbidities and heterogeneity of critically ill patients. Third, mount of preclinical investigations had confirmed the immunomodulatory role of MSCs while none of the reported clinical trials had assessed their effects on immunity-associated biomarkers. Finally, the diagnostic criterion of sepsis is inconsistent and make it difficult to draw scientific conclusions.

The current study will prospectively enrol patients with sepsis induced by pneumonia to exclude the influence of confounding factors. Furthermore, our study will also advance our knowledge of immunoregulatory effects of HUC-MSCs through monitoring and evaluation of cellular immunity-related indicators by flow cytometry. Although our sample size is still relatively small and mortality rate will not be assessed, data on the safety and tolerability of HUC-MSCs in pneumonia induced sepsis will provide pivotal information for the design of subsequent clinical trials in terms of definition of endpoints, key biomarkers and sample size determination.

In summary, the conduct of this study will provide first-hand data and information for clinical evaluation of the safety and efficacy of HUC-MSCs in the treatment of sepsis caused by a single cause (pulmonary origin).

Trial status

The trial is ongoing. Recruitment began on 1 October 2021.

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Contributors CW and DZ wrote the manuscript and contributed to discussion; XB, QY, SJ, CW and DZ participated in the data collection; LT and ZL participated in the design of the study and edited the manuscript. LZ and XZ performed the statistical analysis. All authors contributed to data interpretation and revisions of the manuscript critically for important intellectual content. All authors approved the final version of the submitted manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of work are appropriately investigated and resolved. Roles and responsibilities: Department of Internal Emergency Medicine and Critical Care, Shanghai East Hospital, Tongji University School of Medicine. (Principal investigator: The role is to develop the scientific framework of the study, make final decisions on major issues during the data collection period, analyze data, evaluate and prepare manuscripts for publication). Department of GMP Laboratory of Stem Cell Base, Shanghai East Hospital, Tongji University School of Medicine. (Project manager: Preparation and quality inspection of stem cells for clinical application). Research Center for Translational Medicine, Shanghai East Hospital, Tongji University School of Medicine. (Project manager: The role is to make final decisions on data analysis and prepare manuscripts for publication.

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