BMJ Open

Human umbilical cord mesenchymal stem cell transplantation for the treatment of acute-on-chronic liver failure: protocol for a multicentre random double-blind placebo-controlled trial

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To cite: Wang Y, Li M, Yang T, *et al*. Human umbilical cord mesenchymal stem cell transplantation for the treatment of acute-on-chronic liver failure: protocol for a multicentre random double-blind placebo-controlled trial. *BMJ Open* 2024;**14**:e084237. doi:10.1136/bmjopen-2024-084237

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (https://doi.org/10.1136/bmjopen-2024-084237).

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Received 13 January 2024
Accepted 31 May 2024

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ABSTRACT

Introduction Acute-on-chronic liver failure (ACLF) is a prevalent and life-threatening liver disease with high short-term mortality. Although recent clinical trials on the use of mesenchymal stem cells (MSCs) for ACLF treatment have shown promising results, multicentre randomised controlled phase II clinical trials remain uncommon. The primary aim of this trial is to assess the safety and efficacy of different MSCs treatment courses for ACLF.

Methods and analysis This is a multicentre, double-blind, two-stage, randomised and placebo-controlled clinical trial. In the first stage, 150 patients with ACLF will be enrolled and randomly assigned to either a control group (50 cases) or an MSCs treatment group (100 cases). They will receive either a placebo or umbilical cord-derived MSCs (UC-MSCs) treatment three times (at weeks 0, 1 and 2). In the second stage, 28 days after the first UC-MSCs infusion, surviving patients in the MSCs treatment group will be further randomly divided into MSCs-short and MSCs-prolonged groups at a 1:1 ratio. They will receive two additional rounds of placebo or UC-MSCs treatment at weeks 4 and 5. The primary endpoints are the transplant-free survival rate and the incidence of treatment-related adverse events. Secondary endpoints include international normalised ratio, total bilirubin, serum albumin, blood urea nitrogen, model for end-stage liver disease score and Child-Turcotte-Pugh score.

Ethics and dissemination Ethical approval of this study has been obtained from the Fifth Medical Center of the Chinese PLA General Hospital (KY-2023-3-19-1). All results of the study will be submitted to international journals and international conferences for publication on completion of the study.

Trial registration number NCT05985863.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Patients will be randomly allocated to three groups: one group will receive placebo while the other two groups will receive mesenchymal stem cells either three times or five times.
⇒ This study employs a two-stage randomised grouping design to investigate the safety and efficacy of different courses of mesenchymal stem cells, with the purpose of providing evidence for optimised courses of mesenchymal stem cells.
⇒ The main limitation lies in the inability to estimate the number of patients participating in the second-stage randomisation with the uncertainty of mortality and treatment effect.

INTRODUCTION

Acute-on-chronic liver failure (ACLF) denotes acute decompensation of liver function following acute insults in the context of chronic liver disease. It is primarily characterised by multiple organ/system failures, a high short-term mortality rate and a severe systemic inflammatory reaction.[1–3] Patients with ACLF commonly manifest functional failures in the liver, kidneys, brain, coagulation, circulation, respiratory system and other systems, with 90-day mortality rate reaching up to 58%.[4–6] Currently, effective treatments for ACLF are lacking.[7–10] Liver transplantation stands as the definitive treatment, significantly improving long-term survival. However, the availability of organ sources limits its widespread application,[11–13] necessitating the exploration of new treatment modalities. Given the continuous progress in regenerative medicine, stem cell therapy for promoting liver cell regeneration has emerged as a prominent research avenue.

Mesenchymal stem cells (MSCs) represent a class of pluripotent stem cells derived from the mesoderm and are currently among the most extensively investigated stem cells.[14]

MSCs can be derived from a diverse range of sources and play roles in tissue damage repair, immune regulation and induction of differentiation.[15 16] They secrete various growth factors, cytokines and regulatory factors through paracrine signalling and other pathways. Due to their low immunogenicity, MSCs typically do not induce immune rejection in the host.[17 18] Consequently, MSCs have been widely applied in clinical studies focusing on liver, cardiovascular, renal and neurological diseases.[19–29]

To date, several clinical trials on MSCs for the treatment of ACLF have been conducted.[30] The published studies are summarised as follows: Shi *et al*[31] conducted an open-label, parallel-controlled phase I/II trial. 43 patients with HBV-related ACLF were included, with 24 participants receiving 3 doses of 0.5×10^6 cells/kg umbilical cord (UC)-MSCs treatment, and 19 participants receiving normal saline as a control. Regarding safety, two patients developed self-limited fever within 2–6 hours, which resolved within 12 hours without additional treatment. In terms of efficacy, at weeks 4, 8 and 12, the Model for End-Stage Liver Disease (MELD) scores of patients who received UC-MSCs exhibited a more significant reduction as compared with those of the control group. Survival analysis at the 12-week follow-up indicated that UC-MSCs treatment group exhibited a significantly lower mortality rate compared with the control group (20.8% vs 47.4%, p=0.015).

Li *et al*[32] reported the outcomes of a clinical trial investigating the safety and efficacy of UC-MSCs in treating ACLF, encompassing 45 patients with HBV-related ACLF. The treatment cohort (group A, n=11) received plasma exchange (PE) along with a singular infusion of UC-MSCs totalling 100×10^6 cells administered through the proper hepatic artery while the control cohort (group B, n=34) underwent PE alone. Comparative analysis revealed that, in contrast to group B, group A exhibited significant improvements in albumin, alanine aminotransferase, aspartate aminotransferase, total bilirubin (TB), direct bilirubin, prothrombin time (PT), international normalised ratio (INR) and MELD score 4 weeks post-transplantation ($p<0.05$). Furthermore, at 24 months, group A demonstrated notable improvements in albumin, PT and INR ($p<0.05$). Group A also exhibited a substantially higher cumulative survival rate at 24 months (54.5% vs 26.5%, p=0.015). Importantly, no severe adverse events were reported in any patients.

Lin *et al*[33] conducted an open-label, non-blinded, randomised controlled trial (RCT) involving 110 patients with HBV-related ACLF. The control group (n=54) received standard drug treatment. The MSCs group (n=56) received intravenous infusions of allogeneic bone marrow-derived MSCs (BM-MSCs) at a dosage of approximately $1.0–10\times10^5$ cells/kg once weekly for a total of 4 times, with a 24-week follow-up period. The study reported no infusion-related side effects. Compared with the control group, MSCs treatment significantly improved clinical laboratory indicators, including TB and MELD scores. Throughout the follow-up period, the MSCs group exhibited a significantly lower incidence of serious infections compared with the control group (16.1% vs 33.3%, p=0.04). Moreover, the cumulative survival rate at 24 weeks in the MSCs group was 73.2%, significantly higher than the 55.6% observed in the control group (p=0.03). The findings of this study suggest that peripheral infusion of allogeneic BM-MSCs is safe for patients with HBV-related ACLF, and it can significantly enhance 24-week survival by improving liver function and reducing the incidence of serious infections.

Xu *et al*[34] published an open-label RCT phase I/II trial aimed at exploring the safety and efficacy of PE combined with UC-MSCs transplantation for the treatment of patients with HBV-ACLF. A total of 110 patients with HBV-ACLF were enrolled and divided into the following groups: control (n=30), UC-MSCs (n=30), PE (n=30) and UC-MSCs+PE (n=20). The results showed that the UC-MSCs+PE group had the lowest mortality and adverse outcome rates at 30, 60 and 90 days after treatment; however, the differences were not statistically significant. This research suggests that UC-MSCs combined with PE in the treatment of HBV-ACLF patients are safe but cannot significantly improve the short-term prognosis of patients compared with single-agent treatment. Nevertheless, the long-term efficacy of MSCs requires further investigation.

Schacher *et al*[35] conducted a randomised placebo-controlled phase I/II clinical trial to assess the safety and efficacy of BM-MSCs in patients with ACLF grades 2 and 3. The treatment group received intravenously injected BM-MSCs (1×10^6 cells/kg) five times within 3 weeks while the control group received a placebo. The results revealed that the 90-day survival rate for the placebo group was 20% (1/5), whereas the BM-MSCs group demonstrated a 25% survival rate (1/4). Patients who completed the entire treatment regimen exhibited improved Child-Turcotte-Pugh (CTP) scores, MELD scores and ameliorated ACLF grades. However, the study had a limited number of participants (n=5 in the control group and n=4 in the BM-MSCs group), highlighting the need for larger clinical trials to establish robust scientific evidence.

In summary, based on the current results of MSCs treatment for ACLF, no serious adverse reactions have been reported in terms of safety. Nevertheless, this observation requires validation through large-scale clinical trials. Regarding efficacy, MSCs treatment shows promise in ameliorating short-term liver function and improving patient survival at various time points. However, existing studies exhibit inconsistencies, with most being phase 1 or phase 1/2 clinical trials characterised by small sample sizes and a lack of evidence from multicentre large-sample RCT. Furthermore, the optimal course of MSCs treatment for ACLF remains unclear. Therefore, the objective of this study is to conduct a multicentre RCT to explore the safety and efficacy of different courses of MSCs treatment for ACLF.

Objectives

This study aims to assess the safety and efficacy of distinct courses of UC-MSCs in the treatment of ACLF. The intervention and control groups will be monitored to validate

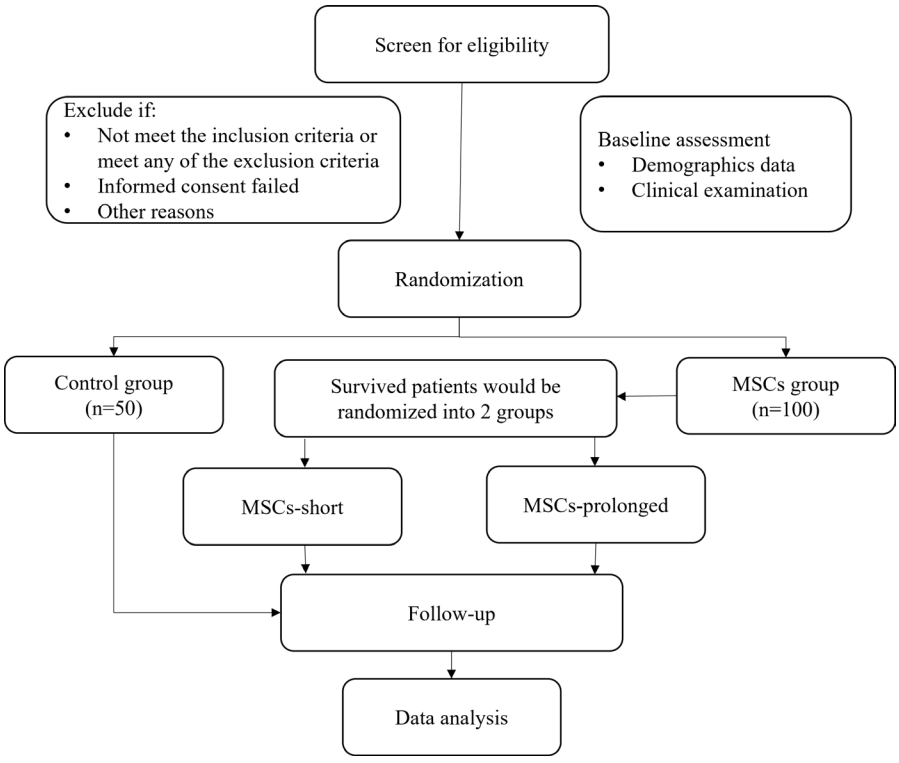

Figure 1 Study flow diagram. MSCs, mesenchymal stem cells.

the impact of UC-MSCs on liver transplant-free survival and other relevant indicators in patients with ACLF. Through the observation of the safety and efficacy of varied UC-MSCs therapy courses, a rational clinical treatment plan can be formulated.

METHODS AND ANALYSIS

Study design

This study is designed as a multicente, double-blind, two-stage, randomised controlled trial. All enrolled patients will receive conventional treatment (CVT). The time of the first infusion of MSCs or placebo will be designated as day 0, and each participant will be followed up for 53 weeks. In the first stage, patients with ACLF will be randomly assigned to two groups at a 1:2 ratio, receiving either three times placebo treatments (control group) or UC-MSCs treatment (MSCs treatment group) at 1-week intervals. After unblinding at the fourth week, surviving patients in the control group will continue to receive CVT and follow-up. In the second phase, surviving patients at the fourth week in the MSCs treatment group will be further randomly divided into two groups at a 1:1 ratio, receiving either two placebo treatments (MSCs-short group) or UC-MSCs treatment (MSCs-prolonged group) at 1-week intervals. A flow diagram of the study is presented in figure 1. Specific treatment details are outlined in the intervention section. The study protocol strictly adheres to the Standard Protocol Items: Recommendations for Interventional Trials checklist.

Patients and enrolment

The patients will be recruited from the Fifth Medical Center of the PLA General Hospital, Beijing Youan Hospital, Shulan Hospital (Hangzhou) and Shenzhen Third People's Hospital. Written informed consent would be obtained from patients or their guardians by a clinical research physician. The planned study start date is 30 September 2023, and the planned end date is 30 December 2028.

Inclusion criteria

1. Age between 18 and 70 years, with no gender limitation.
2. According to the APASL definition, ACLF is characterised as acute liver injury in patients with previously diagnosed or undiagnosed chronic liver disease or cirrhosis, presenting as jaundice (TB levels of 5 mg/dL or more) and coagulopathy (INR of 1.5 or more or prothrombin activity of less than 40%) complicated within 4 weeks by clinical ascites, encephalopathy or both.
3. Voluntary agreement to sign the informed consent form.

Exclusion criteria

1. Patients with acute kidney injury, upper gastrointestinal haemorrhage, hepatic encephalopathy grade II or higher, or uncontrolled infection at baseline.
2. Before the onset of liver failure, previous indicators of the patient, including PLT level less than 50×10^9/L or CTP score greater than 9.
3. Liver cancer or other malignant tumours.

4. Previous or planned liver transplantation within 3 months.
5. Severe organic diseases of the primary extrahepatic organs.
6. Those with a history of venous thrombosis or pulmonary embolism judged by the investigator to be ineligible to participate in the trial.
7. Pregnant, breastfeeding women or those who plan to have a baby in the near future.
8. Highly allergic or a history of severe allergy.
9. Received immunosuppressant and immune enhancer treatment within 1 month.
10. Drug abuse in the past 5 years.
11. Alcohol withdrawal.
12. A history of severe mental disorders within 24 months before screening, including uncontrolled major depression or controlled or uncontrolled psychosis.
13. Those who participated in other clinical trials within 3 months before screening or had previously received stem cell therapy.
14. Other conditions in which the investigator believes the patient is not suitable to participate in this study.

Randomisation and blinding

Randomisation: This study employs a two-stage randomisation. The allocation ratio is decided to be 1:2 in the first stage and 1:1 in the second stage. The patients will be randomly divided into different groups, as previously described, according to a computer-generated randomisation plan. The randomisation in this multicentre clinical trial adopts a competitive enrolment, where each centre uses its own random allocation sequence, employing big stick design as the method.

Blinding: Blinding procedures will be implemented with both investigators and participants. Unblinding envelope will be made for each patient for unblinding or emergency unblinding after blinding. Owing to the particularity of the cell products, drug management personnel will be unblinded. Reagents will be prepared based on the grouping results of the corresponding random numbers in a random coding table. Examinations and quality inspections will be carried out simultaneously. Two unblinded sessions will be conducted. The first unblinded session will be conducted after each participant has completed the infusion and clinical observations in the first stage of the trial. The investigator will assess the participants following the first unblinded session, and eligible participants will undergo the second blinding session. After locking the database at the data review meeting, a second unblinded session will be conducted.

Data collection

The collected clinical data will include demographic information, medical history, treatment history, personal history, allergy history, urine pregnancy test, infectious disease antibody test, autoantibody test, thyroid function test, physical examination, vital signs, abdominal ultrasound, blood routine, urine routine, liver and kidney function, blood ammonia, blood lipids, electrolytes, coagulation function, stool routine, tumour markers, immunophenotyping of peripheral blood cells, CTP score and MELD score, etc. The study schedule and data collection details can be found in online supplemental table 1. All items from the WHO Trial Registration Data Set are detailed in online supplemental table 2.

Intervention

Except for differences in the study drug intervention, all patients will receive CVT according to the treatment guidelines. The placebo to be used in this trial is physiological saline containing 5% human serum albumin, designed to have an appearance similar to that of the MSCs reagent. Both the placebo and cell reagents are packaged in light-proof bags. The MSCs reagent is peripherally intravenously infused, and the dose for each course is 1.5×10^8 UC-MSCs. Intervention details are as follows:

1. Control group: CVT+placebo treatment, including intravenous infusion of placebo at weeks 0, 1 and 2. Surviving patients in the control group, at the week 4 timeline, will continue to receive CVT and undergo follow-up following the completion of three placebo infusions.
2. MSCs treatment group: CVT+UC MSCs treatment, intravenous infusion of UC-MSCs at weeks 0, 1, and 2, and 1.5×10^8 UC-MSCs each time, a total of three times. Patients eligible for secondary enrolment will be randomly divided into MSCs-short group and MSCs-prolonged group.
    1. The MSCs-short group will receive additional intravenous infusions of placebo two times in weeks 4 and 5.
    2. The MSCs-prolonged group will receive additional intravenous infusions of UC-MSCs in weeks 4 and 5, 1.5×10^8 UC-MSCs each time, for a total of another two times.

Briefly, during the entire clinical trial, the control group will not receive UC-MSCs treatment, the MSCs-short group will receive a total of three rounds of UC-MSCs treatment, and the MSCs-prolonged group will receive five rounds of UC-MSCs treatment. The study groups and interventions are illustrated in figure 2.

Outcomes

The primary outcomes are transplantation-free survival rate at 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 8 weeks, 12 weeks, 24 weeks and 53 weeks, as well as the incidence of treatment-emergent adverse events at 0 days, 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 8 weeks, 12 weeks, 24 weeks and 53 weeks after the first infusion of UC-MSCs or placebo. The secondary outcomes are the levels of INR, TB, albumin and blood urea nitrogen at −1 week, 0 days, 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 12 weeks, 24 weeks and 53 weeks, as well as the MELD score and CTP score at −1 week, 1 weeks, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 12 weeks, 24 weeks and 53 weeks after the first infusion of UC-MSCs or placebo.

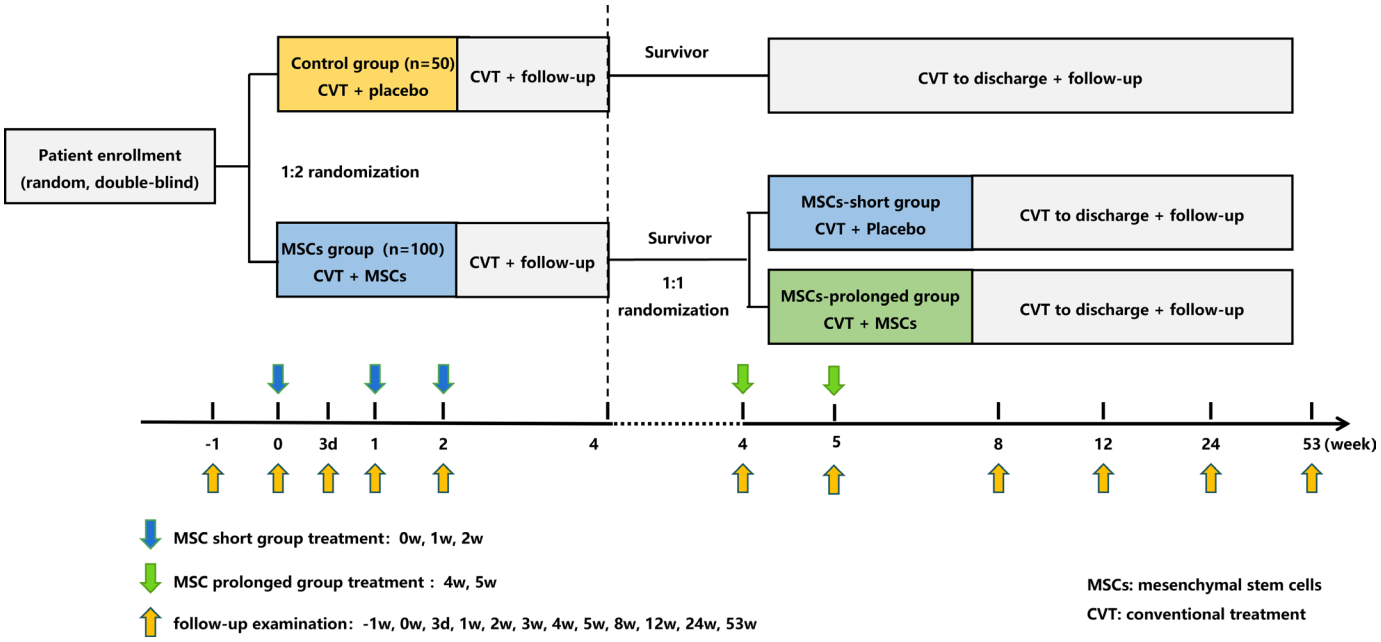

Figure 2 Grouping and intervention diagram.

Criteria for discontinuing

If the following criteria are met, the treatment will be discontinued:

1. The participant withdraws the informed consent.
2. Serious adverse events of participant.
3. The condition of the participant is significantly deteriorated, and it is not appropriate to continue the trial as judged by the investigator.
4. Serious violation of the trial protocol or poor compliance.

In addition, insurance was purchased for the participants, which provides financial compensation in the event of a severe adverse event.

Statistical methods

Sample size

In our prior study,[31] it was observed that, following three administrations of 0.5×10^6 UC-MSCs treatment, the mortality rates for the MSCs and control groups were 20.8% and 47.4%, respectively. Numerous studies have indicated that increasing the dose and frequency of MSCs can diminish mortality in patients with ACLF. Consequently, the estimated mortality rates for the MSCs and control groups in this trial are approximately 20% and 50%, respectively. With α=0.05 (bilateral) and a power of 80%, the calculated sample size is 72 (36 in each group). Considering potential loss to follow-up and the two-stage randomisation of the MSCs group, the targeted sample size is set at 100 cases in the MSCs group and 50 cases in the control group.

Statistical analysis

For the primary outcomes, the intention-to-treat analysis will include all patients who underwent randomisation and medical treatment while the per-protocol analysis will include patients who completed the trial without protocol violations. Descriptive statistical methods will be used to depict baseline characteristics. Quantitative indicators will undergo analysis using the t-test or Wilcoxon rank-sum test and qualitative indicators will be assessed using the χ^2 test or exact probability method. The Wilcoxon rank-sum test will be applied for hierarchical data analysis. The primary outcome, the time-to-event index, will be analysed using the Kaplan-Meier method for liver transplantation-free survival rates, with the log-rank test used for group comparisons. All statistical tests will be two sided unless otherwise specified, and statistical significance is set at $p<0.05$ (two sided).

Data management

In adherence to Good Clinical Practice principles, investigators will maintain detailed original documents of subjects, encompassing trial processes, medication, laboratory test data, safety data and efficacy evaluation on a case report form. Original documents and medical records should be meticulously detailed, clear and easily identifiable. Personal information about potential and enrolled participants will be strictly confidential to the public before, during and after the trial. Original data shall be stored at the institution of the principal investigator and should be audited every year by an independent contract research organisation. Electronic data capture-obtained study data should be stored in an electronic database after locking, with statistical data independently stored by the investigator, sponsor and statistician. Original records shall be accurate, clear, electronically backed up and shall be retained for 30 years poststudy completion.

ETHICS AND DISSEMINATION

The study protocol received approval from the Ethics Committee of the Fifth Medical Center of PLA General

Hospital (KY-2023-3-19-1). Blood, stool and urine samples will be collected for laboratory examinations and scientific research in the current trial and for future use in ancillary studies. All patients participating in the trial would require written informed consent. After enrolment and data analysis, the study results will be submitted to peer-reviewed journals for publication.

Trial status

This trial has not started. The study is estimated to be started on 30 January 2024, and it is anticipated to be completed by 30 December 2028. The estimated study started on 30 January 2024, and the trial finished date will be estimated on 30 December 2028.

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Contributors F-SW, HJ and MS conceived and designed the trial. WY and LM drafted the manuscript. YT and XY revised the manuscript and gave suggestions. All the authors gave final approval of the final version of this manuscript to be published.

Funding This study is supported by grants from the National key research and development plan (2022YFC2304402), the National Natural Science Foundation of China (82070617).

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained from parent(s)/guardian(s).

Provenance and peer review Not commissioned; externally peer reviewed.

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